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RECENT ADVANCES
IN
CHEMOTHERAPY

By

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sary, though with great regret, to divide the present edition into a number of volumes. The first volume thus contains the chemotherapy of scabies and of helminthic and protozoal diseases, with the exception of malaria : the second is devoted wholly to malaria ; the third deals with the chemotherapy of bacterial, rickettsial, and virus infections ; and the fourth contains a survey of sulphonamides and antibiotics, with a discussion of the general principles of chemotherapy. This rearrangement has rendered necessary a complete rewriting of the second edition which must be regarded as now largely of historical interest.

> " Man must pass from old to new
> From vain to real, from mistake to fact,
> From what once seemed good to what now proves the best,
> How could man have progression otherwise ? "

So rapid are the changes that ten years hence it will be surprising if many of the antibiotics now in use are still being employed.

Although the main advance in the past decade has been in the use of antibiotics for the control of bacterial, rickettsial, and some virus infections, very considerable progress, largely stimulated by the War, has also been made in the treatment of helminthic and protozoal diseases. More especially important is the more balanced view now possible of the *rôle* of chemotherapy, in association with other measures, in the control and eradication of malaria and trypanosomiasis in the tropics. The smaller viruses alone resist chemotherapeutic control.

The White Queen was of opinion that " It's a poor sort of memory that only works backwards." It may therefore be hazarded that, if the present rate of advance in chemotherapy continues, within the next hundred years the parasitic infections of man and his domestic animals will have become of little importance and the mortality if not the morbidity which they now cause will have been reduced to almost negligible proportions. Such a change affecting especially the inhabitants of tropical and subtropical countries will have an important bearing on population problems and will intensify the need for an increase in the food supplies available for man. It must, however, be remembered that if Yin can change to Yang the reverse process can also occur and social historians however they may disagree on most points are unanimous that in the past conditions for scientific research have been possible only for very limited periods of time and in particular areas of the civilised world.

Progress may also be hindered by an increasing dominance of drug-fast parasites and the possibilities of mutation among parasites may equal or even defeat the ingenuity of the chemotherapist. Two factors which act as brakes on the progress of chemotherapy may be briefly mentioned. There is at present no wholly satisfactory classification of bacteria : as a result certain bacteria referred to one group by some authorities are placed in another group by others. This obviously leads to considerable confusion when the reactions of bacteria to chemotherapeutic agents are discussed. The second source of confusion is the multiplicity of names given by national pharmacopial committees and by proprietary firms to one and the same chemical compound. Not only may the same compound have many names, but the same name may be applied to two different chemical compounds. Thus in France nivaquine refers to an antimalarial which is called chloroquine in America, where the name nivaquine is given to another compound. An attempt is at last being made by the World Health Organisation to tackle this very complicate problem.

I owe a deep debt of gratitude to the many friends who hav given help and advice and have read the whole or part of thi work at various stages of its production. These include Professo G. A. H. Buttle, E. A. Boulter, Mrs. S. Boulter, Dr. L. G. Goodwi Dr. C. A. Hoare, Miss M. Hollowell, Dr. H. R. Ing, Professor B. (Maegraith, J. S. Morton, Professor J. M. Watson, and Dr. R. Wie S. H. Watkins and my elder daughter Anne have given me inval able assistance in preparing the references and indexes, and n secretary, Miss F. D. I. Rowland, has ably typed the by no mea easy manuscript.

Finally, I must again express my thanks to my publishers for their ever-ready help, courtesy, and understanding.

G. M. FINDLAY.

PREFACE TO THE FIRST EDITION

Recent advances in such a subject as chemotherapy are by no means easily defined. Countless drugs have been introduced for the specific treatment of parasitic diseases, but it is only after constant trials that the value of any compound can be accurately assessed and by this time its introduction can hardly be described as recent. Nevertheless, when such a period as the last ten years is surveyed as a whole, it is apparent that certain definite advances have been made in chemotherapeutic treatment, while in addition to the use of new compounds, a better understanding has been gained of the mode of action of well-established medicaments.

An attempt has therefore been made to describe the more important advances in chemotherapy which have occurred during the post-war period, special emphasis being laid on the fact that the focus of attention has tended to shift from a study of the direct interaction of drugs with infecting organisms to an investigation of the part played by the tissues in chemotherapeutic treatment.

This book could not have been written without the invaluable assistance, which I most gratefully acknowledge, of Dr. C. M. Wenyon, Director-in-Chief of the Wellcome Bureau of Scientific Research, and of Dr. T. A. Henry, Director of the Wellcome Chemical Research Laboratories.

My sincere thanks are also due to Mr. W. H. Gray, Dr. J. W. Trevan, and my colleagues Major H. C. Brown and Dr. J. C. Broom or their kindness in reading proofs and for their constant aid and dvice. The help of Miss I. M. Bellis, Librarian to the Wellcome Bureau of Scientific Research, has also been of the utmost value. I m indebted to Colonel R. B. Lloyd, I.M.S., and the Editor of the *Indian Journal of Medical Research* for permission to reproduce the graphs illustrating the chapter on kala azar, and to the Wellcome Historical Museum for the use of the portraits of Ehrlich, Pelletier, Caventou and Magendie. Finally, I must express to my publishers my appreciation of their courtesy and ever-ready help.

G. M. FINDLAY.

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RECENT ADVANCES IN CHEMOTHERAPY

CHAPTER I

THE HISTORY OF CHEMOTHERAPY

Chemotherapy is that branch of therapeutics which deals with the treatment of parasitic infections by drugs with the object either of eradicating the parasites from the body or of destroying them without damage to the tissues of their host. By a legitimate extension the term chemotherapy may be applied to the drug treatment of malignant growths even though extrinsic parasites such as viruses are not necessarily the causal agents of all tumours. The destruction of invading organisms before they give rise to clinical symptoms as in the prophylaxis of malaria, trypanosomiasis, and streptococcal and venereal infections also comes within the scope of chemotherapy. Chemotherapy in fact is the study of the action of drugs on the relationships of organisms whenever one of these organisms is parasitic on the other.

The word chemotherapy is in many ways unfortunate since it literally means the treatment of disease by chemicals and has thus been used by those who are ignorant of the narrower connotation given it by Ehrlich (1909).* Wright (1927) suggested that "Pharmacotherapy" should replace "Chemotherapy," but even twenty years ago the latter word was already firmly established.

Not every drug used in parasitic infections is necessarily a chemotherapeutic remedy. Acetyl salicylic acid, for instance, if given to a patient with malaria may relieve the headache by reducing the temperature, but as it does not destroy the parasites it is merely a symptomatic remedy. Quinine, on the other hand, relieves the headache and destroys the parasites : it is therefore a chemotherapeutic drug.

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* The earliest use of the word chemotherapy so far traced is by J. P. Diersch who in 1785 wrote "Animadversiones chemicotherapeuticæ de ferro," praes. J. C. Leonardi. Wittebergae.

The history of chemotherapy falls into three phases :—

(1) The period of pure empiricism which ended with the discovery of arsphenamine by Ehrlich and Hata in 1910.

(2) The period from 1910 to the introduction of prontosil rubrum by Domagk in 1935 : in this period protozoal, spirochætal, and helminthic infections only were amenable to chemotherapy.

(3) The modern period associated with the chemotherapeutic control of bacterial infections by sulphonamides and antibiotics.

The belief that the causal agents of disease can be expelled from the body is a very old and very tenacious theory. The intrusion into the body of spirits as a cause of disease dates back to the beginning of recorded things in Egypt and Babylonia and to-day is still widely held by primitive peoples in Asia, Africa, and the New World. One of the earliest and most successful of chemotherapeutic practitioners appears to have been the angel Raphael who, according to the Book of Tobit, expelled the evil spirit which possessed Sara, the bride of Tobias. Tobias " took the ashes of the perfumes, and put the heart and the liver of the fish thereupon and made a smoke therewith. The which smell when the evil spirit had smelled, he fled into the utmost parts of Egypt " —the first use of chemotherapeutic aerosols. Later observers gave a more scientific turn to this idea even before the parasitic theory of disease had become established. R. B. [probably Robert Bostock] (1585) and other followers of Paracelsus believed that disease is caused by impure seeds taken into the body with the food. As long as a person was at unity with himself these seeds were naturally expelled, but when there was a breach of internal concord they had to be ejected by medicines. Millingen (1838), when the *rôle* of the itch mite in scabies had been discovered, said : " Those substances that are known to destroy the insect that produces the itch cure the malady. May not this analogy lead to singular results ? "

Many remedies still in use to-day were originally devised to drive out evil spirits from the body. Sulphur was first employed because its acrid fumes were calculated to annoy any demon so misguided as to take up its abode within the body. Emetics, purges and diaphoretics had a similar origin. Some emetics and some purges did, however, expel parasitic worms, and *Cheno-*

podium album, which is known to have anthelminthic properties, was cultivated in Denmark in neolithic times. In the same way sulphur was recommended for the cure of scabies by Aristotle (384–322 B.C.). Mercury was used at an early stage as an inunction by the Arabs, grey salve and corrosive sublimate being specially valued in the treatment of skin diseases. Rhazes (Abū Bakr Muhammed ibn Zakariyā al-Rāzi, *fl.* 850–932), who, like other Arabian physicians of the time, was wont to test out drugs on animals, was familiar with the toxic effects of mercury from his experiments on monkeys. The Chinese also were cogniscent of the toxic effects of mercury (Chang Pu, *fl.* 860). Mercurial ointment was first prepared by Gilbert the Englishman (*c.* 1180–1250), while Theodoric of Cervia (1205–98), a Dominican who was physician to Pope Innocent IV, vividly described salivation if mercury were continued for more than six days. When in the last years of the fifteenth century syphilis appeared in Western Europe, it is thus not surprising that mercury should have been used almost immediately for the treatment of syphilitic skin lesions. It is not correct to assert that it was mere empiricism which hit on a cure in so short a time (Sherrington, 1946). Though Paracelsus (1493–1541) claimed and is often credited with having introduced mercury into the therapy of syphilis, Theophrastus Bombastus von Hohenheim, the self-styled Paracelsus, was preceded by a number of other physicians (Antonio Benivieni, 1507 (died 1502), Jacopo da Carpi Berengario, 1522 and Bartholomæus Cocles (B. della Rocca), 1504) who all successfully used this drug.

Filings of silver, copper and gold in a boy's urine were lauded by Arnold of Villanova (*c.* 1235–1310), and Bartholomæus Anglicus (*fl.* 1250) is said to have prescribed gold for plague. Arsenic was used mainly as a poison, although from the time of Pliny (Mayerhoff, 1870–98) sulphide of arsenic was prescribed for septic spots on the head, associated with lice. Apart from mercury and antimony, however, very few true chemotherapeutic remedies were in use before the time of Paracelsus and his followers, who gave arsenic, lead, iron, and copper sulphate a permanent place in the pharmacopœia. The most important of really ancient chemotherapeutic remedies are extract of male fern, recommended by Theophrastus (*fl.* 370–285 B.C.), and Galen

(A.D. 130–201); santonin, obtained from *Artemisia maritima* var. *anthelminticum* and esteemed for its anthelminthic action by the Greeks; ipecacuanha root, used for dysentery by the native tribes of Brazil before the Portuguese conquest; infusions of the leaves and seeds of *Chenopodium anthelminticum*, American wormseed, employed by the Aztecs under the name of "apazote." The value of lèche de higueron as an anthelminthic may have been known to negro slaves who were taken to the New World from Africa. The virtues of chaulmoogra oil in leprosy were discovered, according to the Burmese legend, by Rama, King of Benares who, having become a leper, returned to the jungle where he lived on the fruits and leaves of the kalaw or chaulmoogra tree. Having thus cured himself, he one day met in the forest the Princess Pija, also a leper, whom, having cured by the same means, he married. Sushruta, the author of the "Shushruta Samhita," is said to have referred to the value of chaulmoogra, or "tuvaraka," in leprosy.

The majority of drugs were for long selected on purely magical grounds. A real or fancied resemblance to the diseased condition gave rise to the doctrine of signatures which was justified on the grounds of sympathetic magic by the doctrine that like cures like: "Similia similibus curantur." This theory was first given scientific form by Alberti (1734) and later provided the basis for the homeopathy of Hahnemann (1810). Its modern formulation is the curative action of drugs with chemical constitutions closely akin to those of essential cell metabolites. It is erroneous to imagine that the therapeutic properties of cinchona bark were known in South America before the coming of the Spaniards. The romantic story of the Countess of Chinchon has been shown by Haggis (1941) to be without foundation, the bark having at first been exported from South America to Europe as an adulterant of Peruvian bark, *Myroxylon peruiferum*, which early in the seventeenth century had acquired a wholly undeserved reputation as a febrifuge. The bark of the cinchona tree closely resembles that of *Myroxylon*, and there was a widespread belief that medicinal virtues must be inherent in a plant if it resembled one with known therapeutic activities. Blair (1720) in fact proposed to classify medicinal plants on these lines. Until the latter part of last century physicians were still using drugs, chiefly of vegetable origin, the so-called Galenicals, of unknown composition in order to treat diseases of which the cause was likewise unknown. Voltaire added that if possible even less was known of the patient. It is therefore hardly surprising that so few active drugs were known ; rather it is a matter for surprise that so many active substances were actually discovered.

Before chemotherapy could come into being the theory that diseases were due to lack of imbalance of the four humours had to be abandoned, for the humoral theory of ill health was closely bound up with a polypharmacy in which different drugs had qualities which could be so blended as to counteract this humoral imbalance.

As chemotherapy deals with the specific treatment of parasitic infections due to insects, worms, protozoa, spirochætes, bacteria, fungi, rickettsiæ and viruses, it could in addition hardly make much progress till the parasitic theory of disease had been firmly established by the work of Fracastorius (1483–1553), Leeuwenhoek (1632–1723), Pasteur (1822–1895), Koch (1843–1910), and Lister (1827–1912). It was essential also to find methods for establishing infections in laboratory animals under standardised experimental conditions. It was not until the latter part of the nineteenth century that pathology was sufficiently advanced to provide techniques for maintaining and standardising such parasitic infections in animals. Some of the earliest infections thus experimentally produced in laboratory animals were those due to trypanosomes.

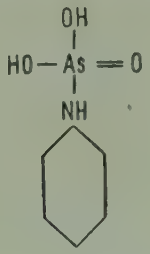
If chemotherapy was not to be restricted to crude vegetable extracts and to inorganic salts the help of the synthetic chemist now became necessary. Once vitalism in organic chemistry had been rejected, not as is commonly believed as a result of the synthesis of urea by Friedrich Wöhler (1828), for Wöhler did not synthesise urea (McKie, 1944), but by the steady accumulation of contradictory facts (Berthelot, 1860), and once the first aniline dye, aniline mauve, had been discovered by W. H. Perkin senior (1858), the way lay open for the fruitful co-operation of the synthetic chemist and the experimental pathologist.

The missionary David Livingstone (1858), somewhere about 1847 or 1848, had been struck with the possibility of giving arsenic, in view of its known tonic action, to horses for the cure of

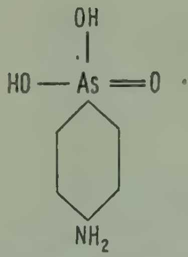
" the tsetse bite," the disease now known as nagana. One horse so treated in Central Africa showed considerable improvement, though it subsequently relapsed and died. Possibly with Livingstone's work in his mind, Bruce, in 1895, after discovering the pathogenic *rôle* of trypanosomes, began to use inorganic arsenicals in infections due to *Trypanosoma brucei.* " It appears," he said, " that arsenic has a specific action, causing the hæmatozoa to disappear from the blood, to hinder or prevent blood destruction and emaciation, and to modify the temperature chart. Whether this drug will completely cure the disease or not remains to be seen." More promising results were obtained in India in surra by Lingard (1899), who conducted experiments with arsenic on trypanosome infections in horses, buffaloes and cows, as well as on the trypanosomes of rats, bandicoots and fish.

In the meantime investigation of aniline dyes, begun in England, had passed to Germany, where Carl Weigert (1876) studied the differential staining of tissues and bacteria, at first with the nuclear dye carmine obtained from cochineal, and later with the aniline dye methyl violet. He also stimulated his cousin Paul Ehrlich, who was particularly interested in the differential staining of the intracellular structures of leucocytes. It was thus but a step to try the effect of dyes in mice infected with trypanosomes. Weinberg's trypan red, sodium 3-sulphodiphenyl-disazo-bis-β-naphthylamine-3 : 6-disulphonate, was shown by Ehrlich (1909) to have a lethal action on *Trypanosoma equiperdum* in mice while its toxicity was low. Unfortunately its curative action on trypanosomes in other animals was small and its use was abandoned. Later, in 1909, Nuttall and Hadwen proved that trypan blue cures canine piroplasmosis though it fails to eradicate the parasites. Ehrlich (1854–1915), who is truly regarded as the founder of chemotherapy, however, showed conclusively that it was possible to destroy parasites without destroying their hosts, and the pessimistic attitude of such pundits as von Behring (1887–88) was therefore once and for all dispelled. Von Behring, as a result of his work on cresols (1888) and on silver cyanide preparations in anthrax (1887), had, it may be remembered, come to regard it as almost axiomatic that the tissue cells of men and animals were many times more susceptible to the poisonous effects of disinfectants than any of the bacteria then known. By 1894, however, hexamethylene tetramine was being administered as a urinary antiseptic (Nicolaier).

Stimulated by the work of Bruce and Lingard, an attempt was made by Laveran and Mesnil, in 1902, to determine the effect of sodium arsenite on infections due to *Trypanosoma brucei* in mice. The drug was again shown to be more toxic for the parasite than the host, but relapses were frequent and the trypanosomes often became resistant to further arsenical treatment. In 1905, however, Thomas showed that an organic arsenical, "atoxyl," was capable of completely eradicating trypanosome infections in mice. This result was surprising, for in the previous year Ehrlich and Shiga (1904) had found atoxyl quite inactive against trypanosomes *in vitro*. To explain this difference in the action of atoxyl on trypanosomes *in vivo* and *in vitro*, a reinvestigation was undertaken of the structure of atoxyl. When first discovered by Béchamp in 1863, atoxyl had been regarded as the anilide of arsonic acid, but observations by Ehrlich and Bertheim (1907) showed that it was really *para*-aminophenylarsonic acid.

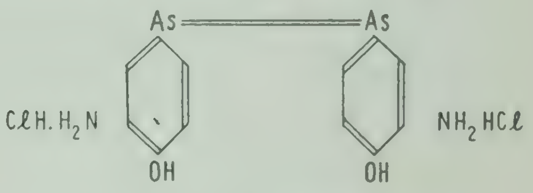


Atoxyl after Béchamp



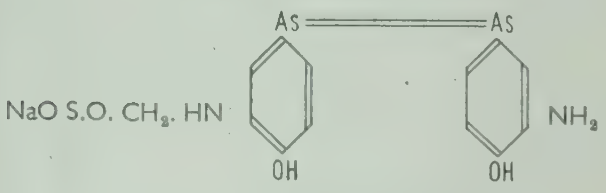
Atoxyl after Ehrlich & Bertheim

Further light on the difference between the trypanocidal activity of the arsenicals *in vivo* and *in vitro* came from the discovery that arsenoxides are active against trypanosomes *in vitro*. Ehrlich therefore came to the conclusion that the tissues must be capable of reducing the quinquevalent arsonic acids to the tervalent arsenoxides before the arsenic can act and the trypanosomes be destroyed. An intensive study of the tervalent organic arsenicals was therefore undertaken. Reduction of the quinquevalent phenylglycine-*para*-arsonic acid with sodium hyposulphite resulted in a tervalent compound, *para*-arseno-phenylglycine. This substance has two arsenic atoms linked by a double bond, each being coupled to the benzene nucleus by a single linkage : it thus represents the arsenobenzene type of organic arsenic and is the precursor of the arsphenamines. The first of these arsenobenzene derivatives, arsphenamine, prepared by Ehrlich and Hata (1910), contained both amino- and hydroxy-groups in the benzene nucleus. Arsphenamine was undoubtedly effective but the toxic reactions it produced were severe and frequent.

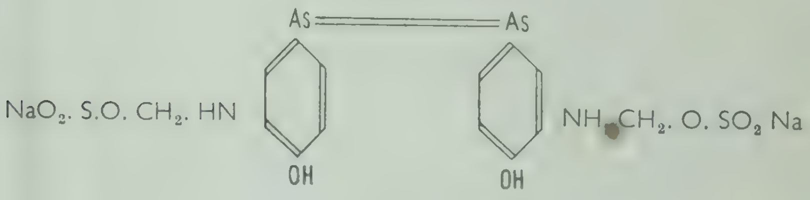


Arsphenamine

Neoarsphenamine and sulpharsphenamine, however, were less toxic and are still in use, though sulpharsphenamine is particularly liable to cause encephalopathy.



Neoarsphenamine



Sulpharsphenamine

In his search for still more potent chemotherapeutic remedies, Ehrlich was influenced by two guiding principles, (1) the idea of a *therapia magna sterilisans*, of a drug which in a single dose would destroy all the parasites in the tissues, and (2) the conception of the chemotherapeutic index.

With certain strains of relapsing fever the arsphenamines may play the part of a *therapia magna sterilisans*, since one or two injections may be sufficient to destroy all the spirochætes and terminate the infection. Penicillin also approaches this ideal.

The chemotherapeutic index of any drug was originally described as the ratio of the minimal curative dose to the maximal tolerated dose :

$$\text{C.I.} = \frac{\text{maximum tolerated dose}}{\text{minimum curative dose}}$$

The greater the chemotherapeutic index of any chemical compound the greater therefore should be its efficiency. The conception of the chemotherapeutic index as a measure of therapeutic efficiency was undoubtedly of great value, but it was soon found that there were considerable difficulties in drawing conclusions from it for the chemotherapeutic index of the same compound may vary considerably on the same parasite in different species of hosts or when administered by different routes even in the same host. Thus from experiments in animals the arsenoxides were at first considered to be too toxic for use in man, though subsequent investigation has shown that they are of value in both syphilis and sleeping sickness. The terms " maximum tolerated dose " and " minimum curative dose " have now largely fallen into disfavour because their errors are large and indefinable ; neither can be determined with accuracy unless large numbers of animals are used. More recent attempts to obtain comparative figures depend on the demonstration by Trevan (1927) that with a limited number of animals accurate comparisons of toxicity can be made only on the 50 per cent. point of response in terms of dosage required to obtain this response. Curves may thus be obtained showing the relationship between the dose administered and the number of survivors. One attempt to provide an accurate index was made by obtaining the ratio of the median lethal and median effective doses (Wien, 1946) :

$$\text{C.I.} = \frac{\text{LD } 50}{\text{CD } 50}$$

where LD 50 is the dose which kills 50 per cent. of animals and CD 50 is the dose which cures the same proportion : but, as

Trevan (1947) has pointed out, such a ratio is of little value if the dose-response curves in any infection are not parallel. In addition, the index does not express the relationship required, a comparison, namely, of the safe dose with the curative dose. A better figure is given by the ratio of the LD 0·1 (the dose which kills all animals except 0·1 per cent.) and the CD 99·9 (the dose which cures all animals except 0·1 per cent.), which can be regarded as accurate substitutes for "maximum tolerated" and "minimum curative" doses. These figures may be determined from the dose-response curves by extrapolation or by calculation from the regression formulæ.

Another index which has been specially used in connection with the testing of antimalarial drugs in animals is the drug equivalent, the dose of quinine necessary to produce the same response, usually the LD 50, as a unit dose of the compound under test (Buttle *et al.*, 1934, 1938). The quinine equivalent has been specially used but a mepacrine or sulphadiazine equivalent has also been employed in testing antimalarials.

During this second period in the history of chemotherapy many quinquevalent arsenicals were investigated and compounds such as acetarsol and tryparsamide came into use. Study of the quinquevalent arsenicals lead on to the synthesis of quinquevalent antimonials which are of value in the cure of leishmaniasis and are superior to tartar emetic in this infection. Tartar emetic was also shown by McDonagh (1915) to be of value in bilharziasis and, despite the introduction of tervalent antimonials such as stibophen, it still retains its place. Other anthelminthics to be introduced were carbon tetrachloride (Hall, 1921) and, later, the far-less toxic tetrachloroethylene (Hall and Shillinger, 1925) for ankylostomiasis.

As chemotherapy arose almost as a by-product of the dyestuff industry, it is not surprising that the earliest large-scale investigations on new remedies should have been carried out in Germany. Suramin was a compound of an entirely new type, first introduced for trypanosomiasis, while for malaria, pamaquin was synthesised, the starting point for this drug being methylene blue, which had been found by Guttmann and Ehrlich (1891) to have a slight antimalarial action. Even as early as 1871 Ehrlich had noted that methylene blue preferentially stains plasmodia in the blood stream at concentrations which leave blood corpuscles and other tissues unstained. Later, German chemists, still fascinated by the similarities of dyes and chemotherapeutic agents, introduced for malaria mepacrine, a compound with an acridine ring. It was the use of mepacrine which enabled the Allies in the war of 1939–45 to carry out successfully the campaigns in Burma and the Pacific.

Finally, still obsessed with the use of dyes, Domagk, in 1935, introduced prontosil rubrum, a compound of sulphanilamide with a red dyestuff which was active against streptococci. Thus the third epoch in the history of chemotherapy was begun.

The demonstration by the Tréfouëls, Nitti and Bovet (1935) that it was not chrysoidin but sulphanilamide which was the active moiety of the prontosil molecule gave wide scope to the synthetic chemist. More than 5,000 sulphonamides and sulphones have now been prepared, and about twelve of these compounds are effective not only in ordinary bacterial infections but against viruses of the lymphogranuloma-psittacosis group, while some of the sulphones have an undoubted action against acid-fast bacilli.

An entirely new field in chemotherapy was opened up by the discovery of Fleming (1929) that a fungus, *Penicillium*, gives rise to a substance which is antagonistic to the growth of a number of bacteria. The work of Florey, Chain, and their colleagues in concentrating penicillin has shown that this antibiotic has a wide range of action, including some viruses. The demonstration that there are several penicillins of different composition and that new penicillins can be synthesised, has great possibilities. The search for antibiotics has now revealed more than eighty different substances obtained from fungi or bacteria with an action on other bacteria. Of these streptomycin, which is active against the tubercle bacillus, is at present the most interesting.

The fact that substances rich in bacteria or moulds would inhibit the growth of bacteria has long been recognised. Fæces rich in *Bacterium coli* was recommended as a dressing for wounds in the Ebers and London papyri.

Although since the time of Ehrlich it had been recognised that protozoa and spirochætes might become resistant to organic arsenicals, it is now known that bacteria and viruses may become resistant against both the sulphonamides and antibiotics. In fact, once its activity has been demonstrated, the most important question to determine in regard to a new antibiotic is how quickly it produces resistant strains.

Many other new chemotherapeutic remedies have also been studied during this third period in the history of chemotherapy. In addition, much has been learnt of the mode of action of chemotherapeutic agents. The conception of Fildes (1940) that chemotherapeutic agents compete with cell metabolites for enzyme systems essential to the metabolism of the cell is a logical restatement in terms of enzyme chemistry of Ehrlich's original conception of cell receptors. The classical example is the inhibition of the action of sulphanilamide by *p*-aminobenzoic acid described by Woods (1940).

Chemotherapy has still many problems to solve. Not all bacterial infections are yet amenable to chemotherapeutic agents, and in the treatment of virus diseases a beginning has only been made. Perhaps the greatest problem, however, is how to prevent the rapid development of resistant strains of parasites in relation to the action of specific chemotherapeutic remedies ; at present this is a true example of John Donne's assertion that " with new diseases on ourselves we war." Yet when it is considered that scientific chemotherapy is less than fifty years old it is perhaps no mean accomplishment that even in that short time it has probably saved more lives than have been destroyed in two of the most destructive wars of history. Much that now appears certain will in the light of further studies have to be modified for, as Charles Darwin noted, " one of the most hazardous of human tendencies is the drawing of general conclusions from limited experience." There is no scientific reason why chemotherapy should not progress until it has banished for ever the hazards to which the human race is subjected by the parasites that beset it.

A study of the five leading causes of death in the United States of America in 1900, 1910 and 1945 already shows that there has been a significant change. In 1900 the main causes of death were (1) tuberculosis, (2) pneumonia, (3) enteritis, typhoid, and other acute intestinal diseases, (4) heart disease, and (5) cerebral hæmorrhage and thrombosis. In 1910 the only change was that heart disease had moved from fourth to first place. In 1945, however, the list had changed profoundly. Hearst diease was still first, but cancer, previously eighth, had gone up to second place, and cerebral hæmorrhage and thrombosis to third. Fatal accidents were fourth and nephritis fifth. While chemotherapy cannot claim the whole credit for the decrease of deaths from parasitic infections and the resulting increase of diseases characteristic of the later years of life, it is entitled to an ever-increasing share of the credit for this remarkable demographic change.

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CHAPTER II

THE CHEMOTHERAPY OF DISEASES DUE TO PARASITIC INSECTS

Many of the insects which feed on man and his domestic animals are ectoparasites since they content themselves with sucking blood and do not multiply within the tissues of their hosts. Such ectoparasites as mosquitoes, ticks, lice, tsetses, sandflies, bedbugs and *Culicoides* can now be controlled by the newer insecticides and repellents, either by spraying or in some cases by the administration of small amounts by mouth so that the insecticide is present in the blood stream in concentrations which are lethal to the insects (pp. 46–49).

More than twenty-five different species of insects, usually in the larval form, are, it is claimed, able to parasitise man by burrowing into his tissues or by living within his excretory channels, thereby giving rise to nasal, aural, ocular, subcutaneous and intestinal myiasis. In temperate climates genuine myiasis is probably most often due to the larva of the lesser house fly and certain tyroglyphid mites. Observations by Hughes *et al.* (1946) and Lyle-Stewart (1946) show that sheep myiasis is controlled by a 0·5 per cent. D.D.T. emulsion or a suspension of 1·0 per cent. gammexane (666); both are superior to proprietary arsenical preparations. For the larvæ of warble flies in cattle, derris root or rotenone is now officially prescribed: the dip must be in contact with the parasites for at least two minutes. In South Africa, creosote is largely used, its action being increased by the addition of alcohol and 0·15 per cent. of sulphuric acid (Mönnig, 1943). Phenothiazine owes its first internal application to the finding by Knipling (1938) that small doses of phenothiazine, given internally to cattle, prevented the development in their fæces of the larvæ of horn flies *Siphona* (*Hæmatobia*) *irritans.* A minimum daily dose of zinc oxide, 1·5 gm. per cwt. of body weight, is also effective (Bruce, 1942). For the majority of these infections, however, as well as for jiggers, due to the chigœ or jigger flea *Tunga penetrans*, for the larva of the Tumbu fly *Cordylobia anthropophaga* Grünberg or for *Demodex folliculorum* there are as yet no known effective chemotherapeutic remedies : the same is true of porocephalosis, due to *Armillifer armillatus.* Whether all forms of tropical eosinophilia are caused by the entry of mites into the lungs is not known, nor is the reason why neoarsphenamine should be of such value in this condition.

For the most common of the insect endo-parasites of man and his domestic animals, *Sarcoptes scabiei,* many chemotherapeutic remedies are available.

SCABIES

There is some uncertainty as to who first described the parasite of scabies, According to Dujardin (1946) it was well known to the Chinese as early as 1800 B.C. Ibn Zuhr, or, as he is more commonly called, Avenzohar (*fl.* 1091–1162), probably confused *Sarcoptes* with the louse but his contemporary, that remarkable lady, St. Hildegard, Abbess of Bingen (1098–1179), gave an excellent description of *Sarcoptes* under the name of snebelza, with instructions how to destroy it (Engbring, 1940). Its true nature was not, however, clearly demonstrated till 1835 when Renucci showed that it was the one and only cause of scabies. This view had also been advocated by G. C. Bonomo (1687).

Nevertheless an efficient remedy had, by then, been known for centuries, since Babylonian physicians were in the habit of treating scabies and other skin diseases with sulphur, an element which from its objectionable smell could be guaranteed to drive out any malignant spirits that had taken possession of the body. If the diagnosis suggested by Hebra (1868) be correct, the successful cure of Naaman, the Syrian, by the waters of Jordan (II Kings, 5) must have been due to their sulphur content.

In an early coptic Papyrus (Papyrus Zoega), sulphur, sodium carbonate and wax are recommended for scabies (Dulaurier, 1843).

In the seventeenth century, despite the current view that scabies was a disease of internal origin, sulphur fumigations were popular, " basting with brimstone," to quote an eighteenth-century diary, being a remedy known to all careful housewives.

A full account of the early history of scabies is given by Friedman (1949).

Although only one new compound, tetmosol, apart from the insecticides, has been introduced during the war years, renewed interest has been taken in the treatment of scabies since its incidence increased in all countries directly affected by the war. As a result, much new information has been acquired of ways to control this condition. The tests of cure for scabies, however, are still somewhat empirical and in most cases have consisted simply of an inspection of the patient immediately after treatment and again four to six weeks later. Only in a few instances has it been proved that *Sarcoptes* has actually been killed by the medicaments applied.

Treatment

Sulphur in the form of the 10 per cent. Unguentum sulphuris B.P., or 5 per cent. for children, has continued in use until the present day, but it has many drawbacks. It is by no means certain in its action, it must be applied on at least three successive days, it has an unpleasant smell, it may cause a dermatitis as severe as the disease itself, and lastly it is messy and damaging to clothes and blankets.

A number of sulphur compounds has therefore been tested while many other compounds have been reinvestigated and their values more accurately assessed. These include dimethylthianthrene, dixanthogen, benzyl benzoate, derris root, rotenone, pyrethrins, D.D.T., gammexane and chlordane.

Many different ways of applying sulphur have been suggested. Flowers of sulphur dusted on the body provide a simple but relatively ineffective means of applying the drug. About 60 gm. are required for an adult but it is usually a race between the death of the parasites and the production of dermatitis. Sulphur lather has been used by Nolan (1938) and Carter (1941), but this method is also relatively inefficient in killing *Sarcoptes*. Sulphur taken internally is valueless. At intervals the use of sodium thiosulphate and hydrochloric acid has become popular. This method involves painting the body, except the face, head and neck, with either a 25 or 40 per cent. solution of sodium thiosulphate, allowing it to dry and then applying 4 or 5 per cent. hydrochloric acid (Parker, 1939). A chemical reaction takes place with the evolution of sulphur dioxide and the precipitation of finely divided sulphur on the skin: the sulphur, nevertheless, is deposited in the form of rather coarse granules. This treatment has the advantage of cleanliness and, as less sulphur is applied to the skin than when an ointment is used, the risk of dermatitis is reduced: to obtain a cure, however, the application must be repeated on at least three successive days. The method, which lends itself to over-treatment, was used in the Army during the war of 1914–18 (Fitch, 1938) and was later popularised by Demianovitch (1937). Ravaut and Mahieu (1934) claim to have cured forty-nine of fifty-two cases; Kulchar and Meininger (1936) treated fifty cases of which one relapsed, but Sheppard (1940) had thirty-two relapses among 210 cases. The treatment has been used with somewhat varying results in veterinary medicine. Chatin (1934) and Stamatin (1937) recorded successful results, but Marotel (1936) reported failure in three cases of sarcoptic mange.

Another supposedly rapid cure is that fully described by Lomholt (1920) as "the Danish treatment of scabies." This makes use of Marcussen's ointment, "**Kathiolan,**" which owes its curative action to its content of potassium polysulphides: these, so it is claimed, give rise to hydrogen sulphide in contact with the skin. The ointment, which must not be spread too thickly, is left on for twenty-four hours, then washed off and the treatment is complete. Lomholt reported the treatment of 678 cases without a single relapse and with only two cases of dermatitis, but Kulchar and Meininger (1936) had four failures in fifty cases, while in fifteen others the application had to be repeated at least once.

Geffen (1944), however, still prefers Marcussen's ointment to benzyl benzoate in the treatment of scabies in children under twelve months of age, although one of the disadvantages of Marcussen's ointment is that it dries up the skin. Clark (1933) and Martin (1938) in America have therefore used the following prescription:—

Vleminckx's sol.	20 per cent.
Camphor less than	1 ,, ,,
Petrolatum	50 ,, ,,
Light mineral oil	10 ,, ,,
Lanolin	20 ,, ,,

After a hot bath and scrub, the ointment is rubbed into all parts except the face and head : two further applications are made at twelve-hour intervals. Twelve hours after the last application the patient washes and changes into clean clothes. Vleminckx's solution (Liq. Calcis sulphurata N.F.) is said to be an alkaline preparation of calcium sulphide and polysulphides. About 2 oz. (60 gm.) of the ointment are required for an adult. The penetrating odour of ointments containing polysulphides and their liability to cause dermatitis are drawbacks to their use. In experiments in rats infected with *Notoëdres* the use of wetting agents appeared to decrease sarcopticidal activity. In North Africa, however, Istin *et al.* (1942) found that the action of potassium polysulphide was enhanced by the addition of ammonium sulphoricinate.

For scabies in cats and dogs Siegel (1949) found calcium polysulphide 1·5 per cent. in propylene glycol, when combined with a wetting agent, of great value.

Dixanthogen (xantoscabin, aulin, aulinogen) was first introduced by Janson (1926) ; it is a light yellow crystalline substance with a slight smell of onions : it melts at 28° C. and is insoluble in water but readily soluble in mineral oils and fats.

$$C_2H_5O\,.\,CS\,.\,S_2CS\,.\,OC_2H_5$$

Dixanthogen.

Janson used two inunctions in twenty-four hours, but the most extensive series of cases has been studied by Haxthausen (1943). No bath is used, but about fifteen minutes is taken to rub in the ointment. After twelve or twenty-four hours the patient takes a bath, washes off the ointment and changes into clean clothes. Among 100 ambulatory patients who used one application of 5 per cent. dixanthogen in vaseline for twenty-four hours there were five relapses. Among 398 ambulatory patients who similarly employed a 10 per cent. dixanthogen vaseline ointment there were twelve, or 3 per cent. of relapses.

Finally, among 152 patients who were given 10 per cent. ointment to rub in just before going to bed, the ointment being washed off twelve hours later, there were two relapses. Among the whole 650 patients there were only two who developed an eczematous dermatitis : these were sensitive to 1 per cent. dixanthogen in alcohol as demonstrated by the patch test. In some cases itching persisted for as long as fourteen days after treatment. This compound is said by Nörr (1929) to be of value in mange in dogs. What is probably the same preparation has been used in Russia by Suteev (1943) under the name of bisethylxanthogen. Results on eighteen children aged eighteen months to twelve years are reported. The compound in 10 per cent. " Vaseline " was rubbed in and left for two to four days, when the patients were bathed. No dermatitis is recorded, but unpublished observations indicate that this reaction is by no means rare.

Dimethylthianthrene (mitigal, mesulphen, sudermo) is dimethyldiphenylenedisulphide, a neutral, yellow, oily liquid, containing 25 per cent. of sulphur : it is odourless and soluble in chloroform and acetone. It was introduced in Germany for the cure of scabies and, with 40 per cent. *iso*heptylacetate, as " odylen " for veterinary use. It was first used for human scabies by Tiefenbrunner (1921), who cured all of 300 cases by, at most, four applications. One case relapsed or was reinfected. Other early reports are those of Sauerbrey (1921), who cured all of sixty-five in-patients, and Kromayer (1921), who cured 120 cases without relapse by five successive daily applications. These writers all emphasise its non-irritant action. Kiess (1922), who developed " a rapid cure," consisting of two applications at an interval of an hour, found that dermatitis could occur particularly if the liquid was not washed off within twenty-four hours. This has been the experience of later observers, who now recommend three applications on successive days after a preliminary bath.

Tetmosol, tetraethylthiuram monosulphide, (thiosan), is a tawny yellow crystalline solid, soluble in the usual organic solvents and melting at body temperature ; it contains 37 per cent. of

$$(C_2H_5)_2N\,.\,CS\,.\,S\,.\,CS\,.\,N(C_2H_5)_2$$

Tetmosol.

combined sulphur. As a pure powder it is not as a rule irritant to the human hand : as a 25 per cent. solution it may stain clothes yellow. The compound was first investigated by Gordon and Seaton (1941) in rats, Syrian golden hamsters, *Cricetus auratus*, and guinea-pigs artificially infected with *Notoëdres* sp. *Notoëdres*, though differing anatomically from *Sarcoptes*, has a very similar life history: it is normally a parasite of cats, rabbits, rats and other small rodents, and very occasionally infects man (Buxton, 1941).

It was found that tetmosol, when mixed with an equal quantity of the methyl ester of the long-chain fatty acids of coconut oil, penetrated the normal skin of the rat's tail within fifteen minutes of having been rubbed in for three minutes, the portals of entry being the hair follicles and sebaceous glands. Benzyl benzoate and dimethylthianthrene in the same strength and vehicle penetrated to a similar depth at an equal rate. When, however, the therapeutic effects of the drugs were tested on *Notoëdres* the percentage of mites affected at the end of twenty-four hours was significantly greater with tetmosol than with the other two compounds. On eggs there was little difference in twenty-four hours, but after seventy-two hours 78 per cent. of the tetmosol-treated eggs were degenerate as compared with 50 and 47 per cent. of those treated with dimethylthianthrene and benzyl benzoate.

In vitro experiments gave similar results, for whereas ten hours' exposure to 5 per cent. benzyl benzoate killed only 10 per cent. of *Notoëdres*, 5 per cent. tetmosol killed 74 per cent.: dimethylthianthrene was more toxic than benzyl benzoate but less active than tetmosol. *Sarcoptes scabiei* var. *hominis*, tested *in vitro*, was rather more resistant than *Notoëdres*; whereas only one of ten mites was killed by a twenty-three hours' exposure to 5 per cent. benzyl benzoate, six of nine mites were killed by 5 per cent. tetmosol in the same time (Gordon and Unsworth, 1943). Oleum sesame, which is said to increase synergically the insecticidal power of pyrethrum and rotenone had no such action with tetmosol.

Reports on the value of tetmosol in human scabies have been made by Gordon and Seaton (1942), Percival (1942), Hagerman and Tottie (1943), Clayton (1943), Bradshaw (1944), Gordon *et al.* (1944) and Wilshaw (1945). Percival made up a 25 per cent. solution as follows :—

Tetmosol	25 per cent.
Polyglycerol ricinoleate . . .	10 ,, ,,
Industrial methylated spirit . .	65 ,, ,,

This solution is quite stable at room temperature, but if kept at lower temperatures tends to crystallise out. Before use, one part of the oily solution is mixed with four parts of water. The 5 per cent. solution is applied without preliminary washing twice daily for three days. Of seventy-nine patients treated in this way all were cured, and one who received 5 per cent. tetmosol in a vanishing cream base was also completely cured : of twenty-five patients who were treated with 0·25 per cent. tetmosol only four were cured.

Hagerman and Tottie (1943), using a preparation of tetmosol known as thiosan, found ten relapses among 500 women and twelve relapses among 200 men, who received one application after having had a bath. When no preliminary bath was used, 1·2 per cent. of relapses occurred.

Clayton (1943) used a similar technique, except that in the preliminary bath a loofah was used instead of a scrubbing brush : ninety-three cases were cured, three were reinfected. A noticeable finding was the rapidity with which itching ceased. Bradshaw (1944) employed a 25 per cent. emulsion of tetraethylthiuram monosulphide and a 20 per cent. cream. The cream and lotion were both diluted with three or four parts of water before use : the cream, however, was messy and did not dry properly. In all, 220 children were treated, the routine being a hot bath, followed by a scrub with the loofah, and the application of the tetmosol. Ten minutes were allowed for the preparation to dry before putting on the clothes. The following results were obtained :—

	Emulsion diluted 1 in 5.	Emulsion diluted 1 in 4.	Cream diluted 1 in 4.
Cured in 1 course . .	89 (85%)	67 (97%)	44 (95%)
,, ,, 2 courses .	13	—	2
,, ,, 3 ,,	3	—	—
Transferred to other treatment . .	—	2	—
Total . .	105	69	46

Thus practically 100 per cent. of cases was successfully cured. Among the 220 patients only two cases of transient erythema but no definite dermatitis occurred.

Gordon *et al.* (1944) used 20 per cent. tetmosol soap and a bath on six consecutive days for six patients, all of whom were cured. Of 110 patients who took three baths with 20 per cent. tetmosol soap, eighty-eight (80 per cent.) were cured, while twenty-two relapsed.

Wilshaw (1945) first employed twice-daily applications of emulsion for three days, a bath being taken before each application. This treatment certainly cured eighteen children and five adults, but was considered to be too time-consuming. On the other hand, the application of the undiluted tetmosol emulsion twice, at an interval of three days, stung too much. The technique which was finally adopted was as follows :—

Adults. Bath and rub in undiluted : twenty-four hours later bath and scrub and put on freshly ironed clothes.

Children. Bath and rub one part of tetmosol and four parts of tap water every twelve hours for three days : then bath and scrub and put on freshly ironed clothes.

In two years 487 patients were treated in the home without supervision. All were cured and there were no reinfections. The only cases of dermatitis recorded are those mentioned by Clayton (1943) and Gordon *et al.* (1944). Clayton (1943) among his ninety-three patients noted seven with a mild dermatitis on the chest and shoulders : this subsided after cessation of treatment. Gordon *et al.* (1944) found that of 242 patients who took three baths followed by 20 per cent. tetmosol soap in six days, only four developed dermatitis and in three of these cases the skin lesions were not due to any sensitivity to tetmosol. Similarly, among twenty-nine volunteers who used the soap for from fourteen to sixty-three days (average thirty-four days) twenty-three reported slight stinging on tender areas, nine reported severe irritation but on continuing found that it grew less, and two had severe reactions, in one case due to the soap. Thus among more than 1,000 children and adults treated with tetmosol the rate of cure has been over 80 per cent., and dermatitis has been so mild as to be negligible.

In animals, Jennings (1942) found that mange is rapidly cured, while Fulton (1943) observed more rapid cures of *Notoëdres* infection with tetmosol than with dimethyl diphenylene disulphide.

A very closely allied compound, tetraethylthiuram disulphide (tenurid, disulfiram), has been used in Scandinavian countries. Hammarskjöld (1943) had one relapse among seventeen patients treated, while Heilesen (1946) noted two relapses among twenty patients. One application only was used, and no preliminary bath.

Benzyl benzoate (Benzylis benzoas. B.P.C., spasmodin, peru scabina, proscabin), $C_6H_5CO \cdot OCH_2C_6H_5$, is a white crystalline substance with a faint aromatic odour and a burning taste. Its melting point is 20° C.; it is insoluble in water but miscible with alcohol, chloroform and ether. It is the active constituent of balsam of Peru, which was used for the treatment of scabies more than eighty years ago (Burchardt, 1869).

Benzyl benzoate was again employed in the treatment of scabies at the beginning of the century by Sachs (1900) and Juliusberg (1901): it was used for some years in Denmark by Nielsen (1917), but although it is said to have been employed by the French army in the early 1930's, it did not, for some obscure reason, become popular in English-speaking countries until 1937, when Kissmeyer (1937) pointed out that in Copenhagen a yearly total of between 3,000 and 4,000 persons had been treated by this method. The original formula required equal parts of soft soap, *iso*propyl alcohol and benzyl benzoate. The *iso*propyl alcohol was soon discarded in favour of industrial methylated spirit, while Currey (1939) and Goldman (1939) substituted anhydrous 20 per cent. soap for soft soap, the irritation and burning caused by the solution being due to the soft soap and alcohol rather than to the benzyl benzoate. Carter (1941) found difficulty in using benzyl benzoate lotion with children owing to the pain when the lotion was applied to raw areas. Erythema of the skin, œdema of the prepuce and scrotum and conjunctivitis were all noted. It is also worth remembering that when made with spirit the lotion is inflammable: patients should not therefore be dried in front of a fire. An emulsion prepared by Touraine and Leroux (1938) is, however, much less irritating. The emulsion is made up as follows:—

Benzyl benzoate	300 gm.	Ethylene glycol distearate	15 gm.
Hydrous wool fat	10 ,,	Water	665 ml.
Methyl cellulose	35 ,,		

A further modification was proposed by Goldman (1939), but with the coming of more stringent wartime conditions a 25 per cent. emulsion of benzyl benzoate in 2 per cent. lanette wax was generally employed. The lanette wax is melted on a water bath, the benzyl benzoate is added and the mixture brought to 60°–70° C. The mixture is then poured into water previously brought to the same temperature, stirred thoroughly and allowed to cool. This preparation has now been widely used and is more efficient than 5 or 10 per cent. benzyl benzoate in spirit or a 10 per cent. emulsion (Mellanby, Johnson and Bartley, 1942). The use of benzyl benzoate in scabies is an excellent example of the old adage that successful treatment depends as much on the way treatment is carried out as on the particular compound used for treatment. Thus certain observers obtain excellent results with one single application ; others find that two applications are essential. A few still believe that a preliminary bath with soap and the use of a scrubbing brush or loofah are absolutely necessary. Yet Mellanby, Johnson and Bartley (1942) have shown conclusively that results with both benzyl benzoate and with sulphur are equally satisfactory with and without a preliminary scrubbing. As Johnson (1943) has emphasised, one essential in treatment is that all parts of the body should be uniformly covered, with special reference to wrists and hands, since these harbour about 63 per cent. of the total mite population (Mellanby, 1943). Unless treatment is given by trained personnel, the patient or his friends are very liable to apply benzyl benzoate only to those parts that itch. If scabies is to be eliminated in a particular family it is also essential to treat all members of the household. It is now realised that from the time of infection to the time when the patient complains of itching there elapses a period of from three to four weeks. Thus for every 100 patients with clinical symptoms between 300 and 400 are in the pre-clinical stage. It is also now agreed that blankets and fomites are only rarely a source of infection, so that their treatment is unnecessary—a saving of endless time and labour when large numbers of scabies patients have to be dealt with.

While even the emulsion of benzyl benzoate may cause pain and burning if applied to open sores, cases of severe dermatitis are rare. A mild and transitory erythema may occur, more especially in children, and itching may continue for some days after cessation of treatment, but severe dermatitis is very exceptional and is as likely to be due to one of the other constituents of the preparation as to benzyl benzoate. Daughtry (1945), using a 25 per cent. emulsion of benzyl benzoate in an aqueous solution of triethanolamine stearate, noted two cases with fine scarlatiniform rashes but no desquamation, one with a papular rash over the extensor surfaces of the extremities, and a fourth with a red, scaly, raised rash on trunk and limbs. Benzyl benzoate followed by lysol baths is liable to cause scrotal dermatitis (Bacon, 1942). Residual itching is easily exaggerated by suggestion.

The following results are illustrative of those obtained by various observers with a single application of benzyl benzoate :—

Number of Cases.	Relapses.	Dermatitis.		Observer.
		Mild.	Severe.	
485	15	14	0	Belbin (1942)
980	8	—	—	Graham (1943)
1,038	14	30	0	Bradshaw (1944)
975	9	20	0	Mallen (1942)
Total 3,478	46	64 (2·56%)	0	

In man, dermatitis is thus rare. External applications show very little toxicity for dogs, cattle, sheep, swine, and horses. Rats, rabbits and cats appear to be far more susceptible to both the irritant and lethal action of benzyl benzoate, the cat tolerating only about one-tenth the dose per kgm. of body weight tolerated by the dog (Graham and Kuizenga, 1945).

Benzyl benzoate dermatitis usually responds readily to rest, avoidance of baths and simple greasy applications such as oily calamine lotion or boric-zinc ointment to which a little arachis oil has been added.

For boils, pustules and secondary bacterial infections, such as impetigo, penicillin ointment is entirely satisfactory : sulphonamides should not be applied. If penicillin is not available, zinc and ichthyol preparations are of use. Calamine lotion should be applied to any areas of residual itching.

The usual routine of applying benzyl benzoate implies painting on the emulsion with a brush. In order to save time in treating large numbers, Hanfling and Goldbloom (1946) used a continuous-pressure hand spray, the face of the patient being covered to prevent injury to the eyes. On the following day the spraying was repeated : twenty-four hours later the patient had a bath and put on clean clothes : 1,266 American soldiers were thus treated without any severe reactions. At first a 25 per cent. alcoholic solution was used, later, when D.D.T. became available, this was incorporated in order to destroy crab lice : the formula was as follows :—

Benzyl benzoate	100 gm.
D.D.T.	10 ,,
Procaine hydrochloride . . .	20 ,,
Ethyl alcohol	1,000 ml.

Of 471 soldiers receiving two sprayings only twelve were unimproved : no reactions were seen.

Hornby (1943) used a 33·3 per cent. solution of benzyl benzoate on puppies with sarcoptic mange. Two applications at an interval of four days were sufficient. Benzyl benzoate and gammexane readily clear up the scabies of gibbons (*Hylobates* and *Symphalangus*), a disease which attacks keepers in zoological gardens (Goldman and Feldman, 1949).

Derris and its active constituent rotenone have from time to time been used in the treatment of scabies in man and animals. Derris (B.P.C.) is made from the dried rhizomes of two leguminous plants, *Derris elliptica* Benth. and *D. malaccensis* Prain, found in Malaya and the East Indies : it is also obtained from the South American cube root *Lonchocarpus nicou* which, under the name of cubé, is said to have long been used as an insecticide by the inhabitants of the Amazon valley.

The main active principle of derris powder is a colourless crystalline substance rotenone, $C_{23}H_{22}O_6$: some sarcopticidal action may be due to deguelin, tephrosin, and toxicarol, as well as to a toxic substance isomeric with tephrosin. Rotenone is insoluble in water but soluble in alcohol, ether and chloroform : it is poisonous if inhaled and is also toxic when given to animals by mouth, death taking place from respiratory paralysis. When applied to the human skin it does not give rise to any generalised symptoms of poisoning.

Derris and its extracts have for many years been used as insecticides in horticultural work, but their employment in the treatment of animal scabies has not been fortunate, since cases of severe dermatitis with toxic absorption have occurred. The toxicity of derris, rotenone and cubé has been exhaustively reviewed by Cutkomp (1943) and Simons (1948).

Ra (1936) prepared a compound which he termed bromorotenone and claimed was non-irritant. Using a 2·5 per cent. preparation in gum arabic, he successfully cured twenty cases of human scabies.

Thomas and Miller (1940) in America, used rotenone in the treatment of twenty-four cases of scabies ; ten received a 1 per cent. rotenone lotion, fourteen a 2 per cent. lotion, with a base of quince seeds or Irish moss, which was applied four times on successive days and is said to be non-irritating : in six cases the course had to be repeated. All cases were cured, but four were reinfected two to four weeks later.

Saunders (1941) employed a suspension of 4 oz. (120 gm.) of derris root powder in 1 gallon of water with 1 oz. (30 gm.) of soft soap. In all, ninety patients with scabies were treated, all grades of severity being noted. No preliminary bath was taken and three applications were made on each of two consecutive days. With the full-strength suspension there was some dermatitis on the penis and scrotum, but when a half-strength preparation was used no dermatitis occurred. In all cases the dermatitis cleared spontaneously within a week. Caller (1941) treated fifty patients varying in age from seven months to seventy-five years with 1 or 2 per cent. rotenone. Three or four applications were sufficient. These results were confirmed by Lunn (1943), who found no dermatitis with the weaker preparation of derris. More severe cases of scrotal involvement have been observed with rotenone than with derris. Mumford (1941) considered the dermatitis very intractable, while Buchan (1941) noted it in three of twenty cases treated with rotenone: no dermatitis was seen in twenty-four patients treated with derris root suspension. Carslaw (1941) found that two and a half days' treatment almost invariably caused a dermatitis: on the other hand, among 250 soldiers treated with 2 oz. (60 gm.) of derris root powder to a quart of water with 1 oz. (30 gm.) of soft soap, scrotal dermatitis occurred in only four, all of whom were red haired (Henriques, 1943). The method used was to take a hot bath and scrub, and then to apply the derris without drying. The derris suspension was heated to 100° F. and painted on with a light brush: the patient then sat in a warm room till dry. The five subsequent applications were made at intervals of four hours. The following results were obtained :—

Severity.	Number of cases.	Relapses.
Mild	150	8
Moderate	72	3
Severe	28	4
Total	250	15

While Mellanby *et al.* (1942), Woodrow (1943) and others have shown that derris and rotenone (2 per cent. emulsion, sarevan) are not as efficient sarcopticidal agents as benzyl benzoate and that four applications of rotenone are necessary to obtain the same percentage of cures as one application of benzyl benzoate, nevertheless derris root is a cheap preparation and is easily obtainable in the tropics, where mass treatment of scabies is often necessary (Williams, 1943). In addition, as Epstein (1942) has shown, it can be applied in cases where sulphur or benzyl benzoate has caused a dermatitis.

All cattle must now be treated with a derris preparation for the control of the warble fly.

Other sarcopticidal agents are **pyrethrins, lethane, xylol, D.D.T., zyclophen, gammexane and chlordane.**

Pyrethrum is prepared from the dried flowers of *Chrysanthemum cinerariifolium* and contains pyrethrins I and II.

Pyrethrin I consists of the ester of chrysanthemum monocarboxylic acid and the keto-alcohol pyrethrolone, while pyrethrin II is the ester of the corresponding dicarboxylic acid. The B.P.C. requires a minimum of 0·4 per cent. of pyrethrin I.

Lemaire and Gaudin (1931) used a gel with 0·5 per cent. pyrethrins, with, it is claimed, success.

An ointment containing 0·75 per cent. of pyrethrins, so that 100 gm. of the ointment represents 83 gm. of pyrethrum flowers, was used by Sweitzer and Tedder (1935) and Sweitzer (1936) in the treatment of scabies. After the usual bath and soaping, ointment was applied all over the body ; on the second night the ointment alone was used, and on the third night a bath was again followed by an application of ointment : 517 cases cleared up in five to seven days. Dermatitis may sometimes be severe (Martin and Hester, 1941).

Angevine (1941) believed that the skin reactions which not infrequently follow contact with pyrethrum are due, not to the pyrethrins, but to the oleoresins. He therefore removed these and dissolved the extract in paraffin. Care was taken to use a bland and anhydrous emollient as the ointment base in order not to destroy the pyrethrins by oxidation. Among 279 patients treated with a preparation known as A200 no case of dermatitis was encountered after three applications. Treatment was quite satisfactory in the absence of baths and scrubbing. Lord and Johnson (1947) also showed that it was possible to separate the pyrethrins from the dermatitis-producing factor.

Mellanby *et al.* (1942) found that A200 killed 94 per cent. of mites, but an emulsion in water containing 2 per cent. of total pyrethrins killed only 87 per cent. of mites while a solution in paraffin containing 1 per cent. of pyrethrin I was fatal to only 71 per cent. when sprayed and 59 per cent. when painted on the body. These results are far below those obtained with a 25 per cent. emulsion of benzyl benzoate or with 10 per cent. sulphur ointment.

Eddy (1944) combined benzyl benzoate and pyrethrins in the same lotion for a concerted attack on scabies, head and crab lice : one application was sufficient to produce a cure.

Roy, Ghosh and Chopra (1941) used 10 per cent. of finely powdered pyrethrum flowers in petroleum jelly for the treatment of sarcoptic mange in dogs and rabbits.

Lethane 384 contains 12·5 per cent. of *n*-butyl "carbitol" thiocyanate* and 37·5 per cent. of β-thiocyanoethyl esters of aliphatic fatty acids by volume in a kerosene base. According to Twinn and MacNay (1943), 90 per cent. of cases were cured by one application of an ointment containing 10 to 50 per cent. of lethane. Although lethane is active in destroying lice, it was found by Mellanby *et al.* (1942) that a 2 per cent. lotion killed only a third of the mites, and a 10 per cent. lotion caused discomfort.

Xylol, with liquid paraffin, has been recommended by Olin (1941), but as it causes intense dermatitis on the scrotum it can be used only in women : its use is also contraindicated in cases with secondary infection or dermatitis.

2, 2-Bis (*p*-chlorophenyl) 1, 1, 1-trichloroethane, or as it is more commonly called **D.D.T.,** in view of its possible toxicity in man, has not been extensively used by itself for the treatment of human scabies, although, as previously mentioned, Hanfling and Goldbloom (1946) incorporated 1 per cent. D.D.T. with benzyl benzoate in a spray and found it of value for eradication of both scabies and crab lice : no toxic results followed its use. When used alone, D.D.T. has failed in many cases to eradicate the infection (Degos and Garnier, 1945 ; Franks and Dobes, 1946 ; Carpenter *et al.*, 1946, and Eddy, 1949). Goldberg (1947) used 7 per cent. D.D.T. in xylol, ether, and petroleum and claims 95 per cent. of cures and no irritation. Elmes (1945) found one application of a 5 per cent. D.D.T. solution in acetone sufficient to cure both sarcoptic and psoroptic mange in rabbits. Nevertheless, D.D.T. is not without its dangers. Hill and Robinson (1945), for instance, report fatal chronic D.D.T. poisoning in two bull terriers, two months after spraying with 5 per cent. D.D.T. in kerosene for demodectic mange. Taylor (1945) has also found a 2 per cent. solution of D.D.T. toxic for rats.

Scabies in man may be readily simulated by the rat mite *Liponyssus bacoti*. D.D.T. solutions kill the lice and anti-histamine preparations allow the lesions to heal (Pirilä and Kilpiö, 1948).

Zyclophen, which has been introduced for the treatment of scabies in children by McElhennye (1946), is made up of equal parts by weight of *o*-phenyl-phenol and 4-chloro-3,5-dimethyl phenyl hydrogen dl-camphorate. To prepare an ointment 1 gm. of each of the above substances is mixed with 4 gm. anhydrous lanoline and 94 gm. of soft paraffin. The ointment is applied night and morning for two consecutive days : no particular precautions are taken in regard to baths or soap. Twenty-two children received treatment and all were rapidly cured. Advantages of zyclophen are said to be its lack of odour, its curative action on secondary infections and the fact that it does not stain clothes.

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* "Carbitol" is a trade name for diethylene glycol.

Benzene hexachloride, or gammexane, the gamma isomer of 1, 2, 3, 4, 5, 6-hexachloro*cyclo*hexane, as a 1 per cent. solution in liquid paraffin has been found by Taylor (1945) to be superior to benzyl benzoate and tetmosol in rats infected with *Notoëdres*. It appears to be of low toxicity (Slade, 1945). Van Everdingen (1948) obtained good results with 193 cases of scabies in Belgium. Touraine (1947) has used a 3 per cent. powder for the treatment of human scabies. About 60 to 80 gm. are required for an adult, 30 to 40 gm. for a child. The powder is carefully applied to all parts except the face and scalp, after which the patient is tightly wrapped in a sheet and put to bed with warm blankets for from four to seven hours. The patient is then dressed in clothes which have not been used for at least four days. Gammexane may also be used as a 1 per cent. preparation in a vanishing cream base. No previous washing is required, and from 15 to 25 gm. must be employed for an adult. After twenty-four hours the patient bathes, changes into fresh clothes and uses clean bed linen. Cannon and McRae (1948) thus treated 100 patients and obtained clinical cures in all. Sixty-one patients required only one application, thirty-six two applications, and the remaining three, three applications. Subjective relief was apparent in two to three hours in a few patients and was complete in from twenty-four to forty-eight hours in at least half. No dermatitis was seen, no patient became sensitised, and secondary infections were not a contra-indication. Similar results were obtained by Wooldridge (1948) and Niedelman (1948).

Gammexane has been used by Draz (1947) for treating *Sarcoptes scabiei* var. *cameli*. The camels are sprayed with a 0·1 per cent. aqueous suspension four times at intervals of ten days. Downing

(1947) also has used a similar preparation with success against *Psoroptes communis* var. *ovis* in sheep. Gammexane is much superior to D.D.T. Apart from the smell of garlic and the slight irritant action on the nasal mucosa, there are no drawbacks to the use of gammexane. Gammexane is much more active against *Psoroptes communis* var. *cuniculi* than D.D.T., but according to Guilhon (1948) it is only twice as active as methyl *iso*propyl 1, 8, dichloro*cyclo*hexane, which may therefore be of value in the treatment of scabies.

Chlordane, $C_{10}H_6Cl_8$, is 5, 8-endo-dichloromethylene-4, 9-dihydro-2, 3, 5, 6, 7, 8-hexachloroindane, and has been developed as an insecticide: it is a viscous, amber-coloured liquid with a mild cedar odour and completely soluble in all the usual organic solvents.

Sarcoptic mange in swine was treated by a spray of 0·25 per cent. chlordane solution: 1 to 2 quarts were needed per pig. Mites disappeared within seventeen days and no systemic disturbances or skin irritation was seen as a result of treatment by one application (Spencer, 1948). In addition to *Sarcoptes scabiei* var. *suis* Magn., two species of mange mites, *S. scabiei* var. *canis* Gerlach and *Demodex canis* Leydig, parasitic in the dog, are susceptible to chlordane. Dogs were dipped in 0·25 per cent. chlordane for from thirty to sixty seconds with at least two complete submergences at 100° to 110° F. Neither chlordane nor gammexane, which was used in the same strength, produced any toxic results in dogs (Muma and Spencer, 1948). Sheep and goats dipped in 1·5 per cent. concentrations of chlordane and cattle sprayed with 2 per cent. concentrations showed, however, toxic symptoms. In acute poisoning the onset is usually sudden, bleating or groaning, grinding of the teeth, opisthotonos, blindness and cyanosis precede death. Subserous hæmorrhages in the large and small intestines and subepicardial hæmorrhages in the heart are common. The liver is usually enlarged and shows some necrosis and fatty degeneration. Care must therefore be exercised in employing chlordane (Radeleff, 1948).

Miscellaneous Compounds. A number of miscellaneous compounds have been investigated by Eddy (1949) on a limited number of human patients. The most promising preparations are the benzyl ether of benzyl salicylate, the methyl ester of

4-*tert*-butylphenoxyacetic acid, the methyl ester of (3-methyl-4-*iso*propyl) phenoxyacetic acid and 1, 2, 3, 4-tetrahydro-2-naphthol-*n*-butyrate. The methyl ester of N-methylanthranilic acid, *p-n*-propoxybenzaldehyde and the *iso*propyl ester of salicylic acid were effective but caused burning or smarting.

Dimethyl phthalate, which is not of great value for human scabies, is said by Osborne (1947) to cure infections in rabbits due to *Notoëdres cati* var. *cuniculi* and *Psoroptes equi* var. *cuniculi.*

Croton-N-ethyl-*o*-toluidine (**Eurax**) has been used in Switzerland by Burckhardt and Rymarowicz (1946), who have treated a series of 326 cases. Eurax is supplied as an ointment containing 10 per cent. of croton-N-ethyl-*o*-toluidine. The method of treatment for ambulant patients is a bath with soap, followed by the application of 50 gm. of ointment, 25 to 40 gm. being used for children and 60 to 80 gm. for very large adults. The face and head are not treated. Twenty-four hours later the same amount of ointment is again applied but no bath is taken. For patients in hospital the initial bath was omitted. Itching disappeared during the treatment period and no cases of eczema were found. Of 140 cases followed up seven showed recurrence, possibly due to reinfection. Eurax also cleared up impetiginous lesions.

Comparative tests by Domenjoz (1946) with eurax and benzyl benzoate, mitigal, tetmosol and dibenzyl disulphide against the rabbit mite *Psoroptes communis* var. *cuniculi* showed that eurax was the most effective since 10 and 5 per cent. concentrations killed all the mites in ten and in from ten to thirty minutes respectively. Eurax is also said to have a more pronounced bacteriostatic effect on staphylococci and streptococci than other drugs used in the treatment of scabies.

Tyrothricin and Benzyl Benzoate. As tyrothricin has been found to be of value in pyogenic infectious dermatoses, Robinson and Robinson (1947a and b) have combined it with benzyl benzoate :—

Tyrothricin	0·05 per cent.
Benzyl benzoate	30·0 ,, ,,
Benzocaine	3·0 ,, ,,
Ethyl alcohol	65·0 ,, ,,
Distilled water, q.s.	

The patients, after a hot bath with soap and water, were told to rub in the solution twice daily from neck to toes ; a second bath was then taken. Seventy-one patients, sixty-five with secondary infections, were treated and all but two were cured. One severe reaction was seen and occasionally complaints were made of mild burning sensations. In sarcoptic mange and mites in domestic animals the same preparation has been used with success by Fleck *et al.* (1949).

A COMPARISON OF SARCOPTICIDAL AGENTS

Up to the outbreak of the second world war comparatively few attempts had been made to compare the relative efficiency of sarcopticidal agents. Some *in vitro* experiments had, however, been carried out on the action of sulphur on *Sarcoptes scabiei* var. *humanus* by placing the parasites on a warm slide and exposing them to concentrations of the drug under investigation. These results, which are fully discussed by Heilesen (1946), are all open to the criticism that the ovigerous female placed on a warm slide finds herself in an entirely abnormal environment. Considerable variations were recorded by different investigators who studied the effects of sulphur. Thus Sachs (1900) found that sulphur required thirty to ninety minutes to cause death, while Silferskiöld (1920) reported that 10 per cent. sulphur ointment was fatal only after twenty-four hours. Lomholt (1921) noted that 25 per cent. of hydrogen sulphide killed *Sarcoptes* in forty-five to sixty minutes.

Gordon and Seaton (1941) showed that *in vitro* both benzyl benzoate and tetmosol killed *Notoëdres*, tetmosol being the more efficient. With *Sarcoptes scabiei* var. *humanus*, however, neither drug was very effective, since three of nine mites survived exposure to 5 per cent. tetmosol for twenty-three hours and nine of ten survived the same exposure to 5 per cent. benzyl benzoate. Heilesen (1946) used an *in vivo* technique for studying the action of sarcopticidal drugs. Ovigerous female mites were placed on the skin of the wrist and allowed to burrow ; after they had entered the skin the preparation to be studied was applied. Twenty-four hours later the mites were removed and examined to see if they were alive. With this technique Unguentum sulphuris 10 per cent., dixanthogen in vaseline, dimethylthianthrene and benzyl benzoate lotion killed all mites within twenty-four hours; thiosan (5 per cent. solution of tetraethylthiuram monosulphide)

THE SARCOPTICIDAL ACTION OF DRUGS TESTED *in vivo*
(Mellanby, Johnson and Bartley, 1942)

Method.	Bath	Scrub.	Cases.	Per cent. cured.	Number of mites dead.	Number of mites alive.	Per cent. mites. killed.
A. **Sulphur.**							
(1) Ung. sulphuris B.P. (10%).	+	+	57	97	269	2	99·5
	+	—	64	90	331	9	97·0
	—	—	26	88	335	8	98·0
(2) Marcussen's ointment	+	—	7	100	169	0	100·0
(3) Flowers of sulphur .	+	—	2	0	4	22	15
	+	—	2	0	26	12	33
	+	—	2	0	29	14	67
(4) Sod. thiosulphate and hydrochloric acid.	+	—	6	0	11	33	25
	+	—	3	0	31	31	50
	+	—	3	66	13	5	72
(5) Sulphur (internally for ten days).	—	—	4	0	0	50	0
(6) Dimethylthianthrene							
Undiluted . .	—	—	6	100	178	0	100
10% in paraffin. .	—	—	3	100	81	0	100
5% in paraffin. .	—	—	5	80	79	1	98·5
2% in paraffin. .	—	—	2	50	62	47	57
0·5% in paraffin .	—	—	1	0	44	21	67
B. **Rotenone.**							
(1) Derris root lotion							
Day 1 . . .	+	—	13	15	121	44	73
Day 2 . . .	+	—	13	46	81	22	79
(2) Rotenone emulsion .	+	—	4	25	559	171	76
C. **Pyrethum.**							
(1) A 200 . . .	+	—	3	33	32	2	94
(2) Emulsion in water (2% total pyrethrins) .	+	—	4	75	41	6	87
(3) Solution in paraffin (approx. 1·7% total pyrethrins)							
(*a*) Sprayed . .	+	—	9	22	91	28	71
(*b*) Painted .	+	—	3	0	93	65	59
D. **Benzyl benzoate.**							
(1) 5% in spirit . .	+	—	5	40	225	23	91
(2) 10% ,, ,, .	—	—	38	95	307	2	99
(3) ,, ,, ,, . .	+	—	64	94	737	6	99
(4) 20% ,, ,, . .	—	—	43	97·5	291	2	99·3
(5) ,, ,, ,, . .	+	—	52	98·0	301	1	99·6
(6) 25% ,, ,, . .	+	—	37	97	175	2	99
(7) Lotion (25% benzyl benzoate: 35% soft soap, 40% spirit) .	+	+	32	100	210	0	100
(8) ,, ,,	+	—	9	100	30	0	100
(9) 10% emulsion .	+	—	12	43	128	16	89
(10) ,, ,, .	—	—	12	75	63	5	93
(11) 20% ,,	3 with and 3 without baths	—	225	99	1,834	2	99·9

killed eighteen of twenty mites, while tenurid (tetraethylthiuram disulphide) killed only nineteen of twenty-nine mites. Of twenty-nine eggs treated with thiosan, twelve were killed and seventeen hatched, while tenurid killed only eight out of forty-three, thirty-five hatching out quite actively. On the other hand, 10 per cent. sulphur ointment killed three of four eggs, dixanthogen four of four, and benzyl benzoate eighteen of twenty eggs. Bradshaw (1944) found in treatment that six out of fifty-eight patients required two applications of benzyl benzoate, and of 218 patients treated with tetmosol eighteen required further treatment : thus there was no significant difference. On the other hand, only two patients treated with tetmosol developed a transient erythema, while none complained of discomfort : all those treated with benzyl benzoate complained of burning and pain, and thirty developed erythema and dermatitis.

The most extensive experiments on the relative efficiency of sarcopticidal drugs are those carried out by Mellanby, Johnson and Bartley (1942) who, after the application of drugs to a particular area of skin, removed all the mites, usually twenty-four hours later, and examined them to see whether or not they were alive. The results (Table p. 37) show that there is little to choose between benzyl benzoate emulsion, Marcussen's ointment, dimethylthianthrene, and Unguentum sulphuris.

Sulphur ointment, on the other hand, is more messy, stains the clothes more readily, and is more liable to cause a dermatitis than the other preparations.

Dobes and Nippert (1943) treated comparable series of patients with sulphur and with benzyl benzoate : in the benzyl benzoate-treated series they encountered no reactions.

Further observations on the relative efficiency of dimethylthianthrene, tetmosol, gammexane, and benzyl benzoate are required especially in relation to their liability to cause skin reactions.

It is curious that, despite the large amount of work which has been carried out on insecticides, we are still ignorant of the way in which any of these drugs act on *Sarcoptes scabiei.*

Prophylaxis

The possibility that certain drugs could be used as prophylactics against infection with scabies has received comparatively little attention. Bacon (1942), however, recommended that anyone sharing a bed with a scabies patient should be painted with benzyl benzoate, and Johnson (1943) suggested that persons in contact with patients suffering from scabies should dip their hands in benzyl benzoate solution. Percival (1942) proposed that the whole population of Great Britain should be treated with sulphur ointment at the same time. Administrative difficulties stand in the way of such wholesale chemotherapy, but Mellanby (1944) has proved that with controlled populations a mass treatment can, in fact, be carried out. Among the female patients of a mental hospital a preliminary investigation showed that 26 per cent. had scabies. On two consecutive dates, therefore, the whole population at risk, 804 in number, was given a single treatment with benzyl benzoate, without a bath. The nursing staff was treated at the same time. No further cases arose in the women's block of the hospital.

It has generally been held that the impregnation of soaps with sarcopticidal drugs is impracticable because of the difficulty in obtaining uniform distribution of the active substance.

The possibility of using a soap impregnated with tetmosol was, however, investigated by Davey *et al.* (1944). It was found that if the tails of clean rats exposed to *Notoëdres* infection were washed once weekly with 5, 10 or 20 per cent. tetmosol soap there was a very considerable reduction in the number of mites lodging on the skin as compared with control rats; the prophylactic effects were more marked with 20 per cent. than with 5 per cent. tetmosol soap. Provided the soap was used twice daily, complete protection was given to rats exposed to constant infection with *Notoëdres*. Later experiments by Gordon and Unsworth (1944) showed that 10 per cent. tetmosol soap applied once daily in the form of a lather protected rats exposed to an intense *Notoëdres* infection for the period of the experiment, sixteen to seventeen days. Two and 1 per cent. tetmosol soaps protected a high proportion of rats, but 5 per cent. tetmosol probably represented the lowest concentration with which consistent results could be obtained (Gordon *et al.*, 1944). Two washings daily with 5 per cent. tetmosol soap were better than 20 per cent. tetmosol soap once a week. On the other hand, a soap with 10 per cent. benzyl benzoate provided no protection against *Notoëdres*.

Gordon *et al.* (1944) then tested the effects on man of washing twice daily with tetmosol soap for long periods. Three volunteers used a 5 per cent. concentration for three months, one a 10 per cent. concentration for one year and one a 20 per cent. concentration for six months without any signs of dermatitis.

Large-scale prophylactic experiments have been carried out by Mellanby (1945) and Bartley *et al.* (1945). The investigations of Bartley *et al.* were carried out on a community of 400 persons whose scabies rate increased during a period of eighteen weeks from 4 to 9·2 per cent. : during these weeks ordinary soap was used. During the next thirteen weeks 5 per cent. tetmosol soap was introduced, with the result that at the end of the period the incidence of scabies was 0·5 per cent. No dermatitis was seen. Mellanby's observations produced similar results. A group of 705 patients with a scabies incidence of 13·2 per cent. used 10 per cent. tetmosol soap for eleven weeks : a similar group of 1,213 patients with a scabies incidence of 1·5 per cent. used a similar non-medicated soap. At the end of the observation period the control group had a scabies incidence of 3·5 per cent., the tetmosol group an incidence of 1·3 per cent.

With the daily use of a soap containing 5 to 10 per cent. of tetmosol it is therefore possible both to guard against the spread of scabies in a community and to decrease the incidence.

Downing (1947) found that 0·1 per cent. suspension of gammexane protected sheep from psoroptic scab for from eighty-two to ninety-six days.

As a prophylactic against scabies, D.D.T. appears to be of little value. Hellier (1945) noted that among soldiers wearing shirts impregnated with D.D.T. or using it for the preceding two months, the incidence of scabies did not differ significantly from that in a control series.

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EXTERNAL BLOODSUCKING PARASITES AND CHEMOTHERAPY

If chemotherapy be defined as the study of the action of drugs on the relationship of the host to its parasites, it may be extended to include not only intrinsic parasites which invade the tissues of their hosts but those which live by sucking blood. Brief mention must therefore be made of the use of chemotherapeutic drugs administered to animals to destroy bloodsucking parasites.

Attempts by Parman *et al.* (1928), Bruce (1940), Emmel (1942) and Creighton *et al.* (1943) failed to demonstrate control of external parasites by internal medication. Investigations by Knipling (1938) and Bruce (1939, 1942) demonstrated that phenothiazine and zinc oxide fed to cattle would destroy the larvæ of the horn fly, *Siphona irritans* L., breeding in the fæces. In 1944 Lindquist *et al.* showed that bed bugs, *Cimex lectularius* L. and *C. hemipterus* F., can be killed by feeding them on rabbits which have been given sub-lethal doses of D.D.T. and pyrethrum. Some toxic action was also exhibited on *Stomoxys calcitrans* L. which had engorged on rabbits given pyrethrum. The dosages required to produce insecticidal action in the blood were relatively high, usually over 300 mgm. per kgm. of body weight. Later De Meillon (1946) demonstrated that *Aëdes ægypti*, *Cimex lectularius* and *Ornithodorus moubata* Murray were killed or injured when fed on a rabbit which had been given gammexane. Garnham (1947) obtained similar results with *A. ægypti* fed on gammexane-fed rabbits. Knipling *et al.* (1948) investigated the effects of a number of compounds on body lice, *Pediculus humanus var. corporis* Deg., *A. ægypti*, the rabbit-ear mite *Psoroptes equi* var. *cuniculi* Delafond, and the tick *Amblyomma americanum*.

Lice were found to have been killed after one blood meal on a rabbit given 300 mgm. per kgm. or less of the following drugs :—

Gammexane ;
2-Caproyl-1, 3-indandione ;
Chlordane ;
Potassium salt of 2-*cyclo*propyl-carbonyl-1, 3-indandione ;
Chlorinated camphene ;
2-Enanthyl-1, 3-indandione ;

2-*iso*Valeryl-1, 3-indandione ;
Sodium salt of 2-*iso*valeryl-1, 3-indandione ;
2-Pivalyl-1, 3-indandione ;
Sodium salt of 2-pivalyl-1, 3-indandione ;
Dicoumarol ;
Powdered sabadilla seed.

Aëdes œgypti were killed when allowed to feed on rabbits given 300 mgm. per kgm. or less of the following drugs :—

Gammexane ;
Chlordane ;
Potassium salt of 2-*cyclo*propylcarbonyl-1, 3-indandione ;
2-*iso*Valeryl-1, 3-indandione ;
Sodium salt of 2-*iso*valeryl-1, 3-indandione ;
2-Pivalyl-1, 3-indandione ;
Chloromethyl *p*-chlorophenyl sulphone ;
Dicoumarol.

Ear mites were killed by single doses of gammexane given to their hosts at the rate of 100 to 300 mgm. per kgm. Doses of 50 mgm. per kgm. caused death of only some mites.

Larvæ and nymphs of *Amblyomma americanum* were not affected when fed on a rabbit given an oral dose of 25 mgm. of gammexane per kgm. 2-Pivalyl-1, 3-indandione given to a rabbit at the rate of 25 mgm. per kgm. prevented engorgement of all larvæ and most of the nymphs, but 5 mgm. per kgm. proved ineffective. There is little relation between the value of some of these chemicals as contact-insecticides against lice and mosquitoes and their value as chemotherapeutic agents. As contact-insecticides against lice, D.D.T., benzene hexachloride, chlordane, and chlorinated camphene are about as effective as 2-pivalyl-1, 3-indandione, but unfortunately the question of toxicity must also be taken into account. A method of estimating gammexane in the blood of cattle is described by Barlow (1947). The blood is oxalated, ground with anhydrous sodium sulphate, and extracted with benzene : the gammexane is de-hydrochlorinated and estimated by chloride determination. After a dose of 0·25 gm. per kgm. the blood concentration is from 1 to 2 mgm. per 100 ml. for twenty-four to forty-eight hours.

Indandione derivatives cause hæmorrhages due to hypoprothrombinæmia (Kabat *et al.* 1944), and D.D.T. is also not without toxicity.

So far as is known, extrinsic chemotherapeutic agents show a high degree of specificity. 2-Pivalyl-1, 3-indandione is highly effective against lice and relatively ineffective against mosquitoes, whereas gammexane is much more effective against mosquitoes than against lice.

The use of insecticides against bloodsucking ectoparasites raises problems of considerable importance. It has long been known that both lice, fleas and ticks refuse to feed on certain persons. Mr. Pepys, it will be remembered, when staying at Portsmouth in 1662, was delighted because fleas refused to bite him while attacking his bed-companion. When lice have been fed in large numbers on the same person for some weeks they begin to die, possibly because of the lack of some trace element in the blood. Certain persons may have toxic substances in their sweat. Brennan (1947) has recorded the case of a white American whose sweat was so lethal to ticks that when they fed on him they fell off dead.

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CHAPTER III

THE GHEMOTHERAPY OF HELMINTHIC INFECTIONS

The most primitive chemotherapy of which there is record was undoubtedly concerned with the finding of remedies for the larger intestinal worms, which form an example of parasitism so obvious that their presence in the fæces cannot have failed to attract attention at an early stage of man's history. *Chenopodium album*, for instance, was cultivated in Denmark from *c.* 1500 B.C. onwards by peoples in the neolithic stage of civilisation.

Among the many hundreds of vegetable substances formerly used as anthelminthics, most are now of historical interest only: a few, such as male fern, which was recommended by Theophrastos (370–285 B.C.) and Galen (A.D. 130–201) still retain their old prestige. Unripe pomegranates, crushed and steeped in strong wine, were recommended by Cato the Censor (234–149 B.C.): Italian peasants still use pomegranate bark.

Hall (1928) divides the study of anthelminthics into three periods. The first was characterised by the purest empiricism, when any drug which would remove some worms was classed as a vermifuge, without consideration of the number of worms that remained behind. This era of uncritical medication came to an end with a recognition of the economic importance of hookworm disease. In 1880, male fern was use by Perroncito for the treatment of hookworm disease, thymol by Bozzolo in 1881.

In the latter year Bäumler reported unfavourably on the efficiency of oil of chenopodium, a remedy originally used by the Aztecs. Thirty years had to elapse before Schüffner and Vervoort (1913) demonstrated its real value. Meanwhile Bentley (1904) had found β-naphthol satisfactory and, with a growing realisation of the good results obtained by thymol, male fern passed out of fashion for the treatment of ankylostomiasis.

This second period of critical empiricism was characterised by an increasing tendency to test the absolute and relative efficiency of the various anthelminthics used for the removal of hookworms. The evacuation of worms and clinical improvement were naturally the first criteria used in formulating judgments on drugs. Unfortunately the numbers of worms expelled tell nothing of the numbers that remain, while clinical improvement is impossible to assess mathematically.

Numerous efforts were made to devise egg-counting methods (Stoll and Hausheer, 1926 ; Lane, 1932 and 1935). Even though Lane (1935) claimed that by direct centrifugal floatation pushed to finality it is possible to detect a single normally ovipositing hookworm, it must be remembered that certain anthelminthics may temporarily inhibit the egg-laying capacity of worms, full activity being recovered after a shorter or longer period (Maplestone and Mukerji, 1933). Egg counting is not now considered to be a reliable method of assessing intensity of infestation or anthelminthic action, since the egg-count is affected by many factors, varies considerably even when other circumstances are kept as constant as possible, and in any case indicates only numbers of female worms. More accurate evidence of anthelminthic efficiency was obtained by using a more or less standard treatment following a test treatment, the worms removed on both occasions being identified and counted. This method, which is directly applicable to man, has given information of value but it lacks exactness when the relative efficiency of various anthelminthics is to be compared, since it presupposes that the number of worms harboured at the beginning of each experiment is approximately the same.

The third epoch dates from the year 1915 when, in the United States, Hall and his colleagues introduced a critical method for testing anthelminthics (Hall, 1928). This was not the first time that critical testing had been attempted, for Perroncito (1880) and Grassi and Calandruccio (1884) had employed critical testing in their studies on liver fluke disease of sheep, when they established by post-mortem examination that male fern can kill liver flukes. Both as a practical proposition and as a scientific procedure their work was neglected for a third of the century.

The method of critical testing adopted by Hall consisted in administering known doses of drugs to animals of various species, collecting all worms from the fæces for at least four days, some-

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times for weeks or months, killing the animals and at autopsy collecting, identifying, and counting all the worms present. By this method such important anthelminthics as carbon tetrachloride, carbon tetrachloroethylene, carbon trichloride or hexachloroethane, and hexylresorcinol have been discovered.

The method, however, is open to criticism on several counts. It cannot, of course, be used on man or in small trichostrongylid nematode infections in animals : in comparing the efficiency of different anthelminthics it must be remembered that closely allied species such as *Necator americanus* and *Ancylostoma duodenale* are not equally susceptible to a drug such as hexylresorcinol : an accurate mathematical comparison of the efficiency of different anthelminthics can be obtained, as Carr-Fraser (1935) has pointed out, only by comparing the residual infestation of each of the treated hosts with the initial infestation of each of the untreated hosts for which purpose special equations must be used. The most important source of error, the digestion and disappearance of worms killed by the drug in the intestine, has been investigated by Sprent (1946). By placing *Bunostomum trigonocephalus* directly in the duodenum of sheep it was found that while some were expelled many were rapidly digested and were thus not found in the fæces : if the worms are killed they are quickly digested, so that while judgment on the action of a highly efficient anthelminthic would not necessarily be vitiated, a moderately efficient anthelminthic might easily be regarded as of little value.

The examination of anthelminthic action on worms *in vitro* is obviously a very crude procedure which may lead to serious error in assessing anthelminthic efficiency. A more refined *in vitro* method, however, is the study of worms *in situ* in the perfused intestines of laboratory animals. By this means Rogers (1944) found that the efficiency of hexylresorcinol was reduced by half in 1 per cent. sodium tauroglycholate, while the adsorption of hexylresorcinol on mucus prevented its penetration into worms under the mucus. Thus the lack of activity of hexylresorcinol against *Nippostrongylus muris* in the rat can be accounted for by the inhibitory action of bile and mucin, a conclusion at which Trim (1944) also arrived from *in vitro* experiments. Tetrachloroethylene, on the other hand, is not inhibited *in vivo* by bile or mucus: in fact, it appears to stimulate *Nippostrongylus* to leave the shelter of mucus.

Despite the comparatively crude methods available for investigating anthelminthic efficiency, progress still continues in the discovery of new anthelminthics. This is specially true in the field of dyestuffs. For some years gentian violet has been used for treating *Strongyloides stercoralis* and *Hymenolepis nana* in man: more recently phenothiazine has become established for the treatment of certain helminthic infections of domestic animals.

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COMPOUNDS OF ANTIMONY

Antimony sulphide, as kohl, has for centuries been applied to the eyes by primitive peoples in Africa and Asia; it was recommended for disease of the eye in the Ebers Papyrus, which dates from at least 1500 B.C.

Antimony was extensively employed medicinally by Arabian physicians; it was enthusiastically praised by Paracelsus, by Gesner (1555), and by the pseudo-Basel Valentine, in reality Johann Thölde, whose "Triumphwagen des Antimonii," published early in the seventeenth century, included material from an earlier period. The early history of antimony is very fully discussed by Habeck (1936). Tartar emetic was first described in 1631 by Adrian von Mynsicht, although it had by then come into use for the treatment of pulmonary diseases; during the sixteenth century its employment was attacked, not because of its toxicity, but because it had not been sanctioned by Galen. In 1906 Broden and Rodhain showed that tartar emetic could be given intravenously without mishap, but the introduction of antimony into chemotherapy dates from the discovery by Plimmer and Thomson (1908) that the injection of tartar emetic, or the analogous sodium salt, into rats and mice suffering from trypanosomiasis, causes a rapid disappearance of trypanosomes from the peripheral blood stream.

Antimony now has an established place in the chemotherapeutic treatment of schistosomiasis, leishmaniasis and animal trypanosomiasis: in addition it is of some value in filariasis and lymphogranuloma venereum; its action in granuloma inguinale is less certain.

In the treatment of helminthic infections with antimony both ter- and quinquevalent compounds have been investigated.

Estimation

Several quantitative methods have been elaborated for the estimation of antimony in biological materials in order to control dosage and avoid toxic symptoms.

Qualitative tests for the presence of antimony in the urine (Di Cristina and Caronia, 1916; Mallardi, 1921) were soon replaced by quantitative methods based either on the colorimetric estimation of antimony as sulphide (Brahmachari, Das and Sen, 1923; Khalil, 1931) or on the Gutzeit test (Boyd and Roy, 1929). Modifications of the Beam and Freak (1919) method and of the Gutzeit test are both time-consuming and inaccurate for small quantities, while the colorimetric test for pyrocatechol (Khalil, 1936a and b) is applicable only to stibophen (fouadin) and assumes that the urinary content of antimony runs parallel to the excretion of pyrocatechol, an assumption which Goodwin and Page (1943b) have shown to be without foundation.

Three recent advances have, however, been made in methods of estimating antimony in organic materials: (1) the use of the polarograph; (2) the rhodamine B test; (3) the use of radioactive antimony. The polarographic determination of antimony, described by Page and Robinson (1942), is based on the principles of analysis with the dropping mercury electrode laid down by Kolthoff and Lingane (1941), Müller (1941), and Kolthoff (1942). Page and Robinson (1942) showed that in the presence of normal hydrochloric acid the diffusion current for tervalent antimony is directly proportional to the concentration over the range 0·05–0·0001 per cent. antimony. Tervalent but not quinquevalent antimony in N/1 hydrochloric acid has a half-wave potential versus the saturated calomel electrode of — 0·15 v.: as a result tervalent antimony can be determined directly in the presence of the quinquevalent form.

Goodwin and Page (1943a and b) applied the polarographic method to the detection of ter- and quinquevalent antimony in the urine and blood of mice and men. Under favourable conditions, the error does not exceed ± 2 per cent. over concentrations ranging from 10^{-1} to 10^{-3}, ± 5 per cent. over the range 10^{-3} and 10^{-4}, and ± 10 per cent. over the range 10^{-4} and 10^{-5} gm/100 ml. While estimations of antimony in plasma and urine are not subject to any gross error, the presence of certain as yet unidentified substances in tissue digests leads to polarographic curves difficult to interpret and not necessarily reproducible.

Gellhorn *et al.* (1946) have used the rhodamine B method of estimating antimony, originally introduced by Frederick (1941) and modified by Maren (1945). This method has the advantage of requiring no elaborate apparatus and thus of being of use in routine clinical examinations; in addition, it can be applied not only to plasma and urine but to tissue extracts, and is applicable in a range suitable for distribution studies. Its disadvantage is that the exact composition of the coloured rhodamine B antimony complex is not known so that neither the kinetics nor the optimal conditions of the reaction can be adequately studied. This leads to empiricism in the technique and probably accounts for the not inconsiderable error in precision. The calibration curve from 0 to 2 μgm. is rather poorly defined and the accuracy in this range is less than in the range 2 to 15 μgm. No differentiation can of course be made between ter- and quinquevalent antimony.

Maren's method in its turn has been modified by Lippincott *et al.* (1947a) by using chemicals of the proper degree of purity and by reading the optical density of the unknown against reagent blanks instead of against benzene. Iron is eliminated by precipitating the iron as ferric-ferrocyanide.

Cowie *et al.* (1945), Brady *et al.* (1945), Smith *et al.* (1945, 1946a and b), Lawton *et al.* (1945) and Bartter *et al.* (1947) have used radioactive antimony in the form of tartar emetic, an aqueous suspension of antimony trioxide and sodium antimonyl xylitol. With radioactive antimony, in the range of from 0·25 to 40 μgm., the maximum deviation was ± 3 per cent. with an average deviation of 1 per cent.

The Pharmacology of Organic Antimonials

A study of the distribution of antimony by the more refined methods now available has not revealed any very new or startling facts. Brady and his colleagues (1945) and Cowie *et al.* (1945) found that in dogs, after a single intravenous dose of either radioactive tartar emetic, a suspension of antimony trioxide or sodium antimonyl xylitol, a rapid fall in the concentration of antimony in the blood occurred during the first hour, followed by a slower rate of removal for the next four to sixteen hours. Sometimes a slight rise again occurred in the blood concentration twenty-four to thirty-six hours after the injection. When dogs were killed thirty-six hours after injection of tartar emetic, an examination of dried tissues from the different organs showed that the liver contained the greatest concentration of radioactive antimony while the combined thyroid and parathyroids had the next highest concentration, closely followed by the dead bodies of *Dirofilaria immitis*. After twelve intravenous injections of sodium antimonyl xylitol, containing 0·8 mgm. of antimony per kgm. of body weight, the thyroid gland contained the largest amount per unit weight, followed by the liver, parathyroids and dead worms. It is possible that these tissues have a specific affinity for antimony since their antimony content was far greater than could be expected of the random distribution of a soluble compound throughout the body. Concentrations of antimony in the skin and lymphoid tissue were low, suggesting that large amounts of antimony would be necessary to destroy *Wuchereria bancrofti* or *Onchocerca volvulus*, unless these worms have a specific affinity for antimony.

The effect of injecting hamsters suffering from *Schistosoma mansoni* with radioactive antimony has been investigated by Smith *et al.* (1945). In hamsters, as in dogs, the concentration of radioactive antimony in the thyroid is large.

Human experiments have been carried out by Smith *et al.* (1946a and b). In man, after a single intravenous injection of tartar emetic (0·8 mgm. antimony per kgm. of body weight) the concentration curves of antimony in the blood and blood fractions were reduced within one hour to 50 per cent. and after thirteen hours to 1 per cent. of its initial value immediately after injection. Total excretion curves show that probably 90 per cent. of the initial dose is excreted by the kidneys, the remainder in the fæces.

External measurements with a Geiger counter over regions adjacent to organs within the body enabled a rough estimate to be made of the amount of antimony in the various organs, as shown in the table.

ESTIMATION OF ANTIMONY CONTENT OF ORGANS IN MAN AS DETERMINED BY EXTERNALLY DETECTABLE RADIOACTIVITY (Smith *et al.*, 1946b).

Organ.	Microgram equivalents of antimony at various times after intravenous injection.				
	168 hours.	13 days.	20 days.	23 days.	30 days.
Thyroid	9·6	18·1	8·9	7·1	6·1
Gall bladder . . .	42·1	42·8	50·2	44·9	24·2
Liver	40·0	47·7	39·2	31·1	26·2
Leg	3·6	2·5	2·5	3·7	3·1
Cranium (parietal region) .	—	2·5	—	—	—

The concentration of radioactive antimony in red cells as compared with plasma showed a fairly constant ratio of about 5 : 1.

In man very similar results were recorded by Bartter *et al.* (1947) following a single intravenous injection of radioactive antimony. For the first twenty-four hours there was rapid excretion, 80 per cent. of the antimony being in the urine, 20 per cent. in the fæces. For the ensuing five days the rate of elimination was slower, for whereas 12 per cent. of the antimony was excreted in twenty-four hours only 30 per cent. had been eliminated in seven days.

Gellhorn, Tupikova and van Dyke (1946) studied the distribution of antimony in the hamster after the administration of tartar emetic, anthiomaline, stibanose and neostibosan, using the rhodamine B technique. After a single injection, antimony became concentrated in the liver, the concentration being most marked in the case of the tervalent compounds. After seven daily injections of tartar emetic or stibanose the amount of antimony in the liver showed an absolute increase but the percentage of the total dose of antimony administered did not increase in the case of stibanose and was even smaller in the experiments with tartar emetic. Only traces of antimony were present in the spleen after injections of tartar emetic and anthiomaline, but with neostibosan and stibanose the concentration in the spleen was considerable.

When toxic doses of antimony preparations were administered no great accumulation of antimony was found in such organs as the lungs, heart or brain. If the value of 1 be taken as the concentration of antimony in the liver at the time of death of animals receiving toxic doses of tartar emetic, then the antimony concentration at a comparable time in the livers of hamsters receiving

Anthiomaline	was	3·6
Stibanose	,,	8·6
Neostibosan	,,	44·0

These figures show that there is no critical concentration of total antimony required to produce death. In a man who was killed sixteen days after receiving 0·274 gm. of antimony in the form of tartar emetic the amount of antimony in micrograms per gm. of tissue, according to Smith *et al.* (1946b), was

Liver	11·6
Heart	2·4
Kidney	2·5
Brain	0·95

Estimations by the rhodamine B test (Gellhorn, Krahl and Fertig, 1946) showed that 50 per cent. of the tervalent antimony of tartar emetic and anthiomaline is excreted by the intestine into which it is passed from the bile twenty-four hours after a single intra-peritoneal injection. Quinquevalent compounds were excreted in the intestine only to the extent of 3 to 6 per cent., the main excretion taking place in the urine. Lippincott *et al.* (1947a), using a modification of Maren's method, found that the antimony concentration in the red blood cells was invariably greater than in the plasma. For several hours after administration patients given tartar emetic had higher blood concentrations than had those given stibophen. Small amounts of antimony were still being excreted in the urine 100 days after the end of a therapeutic course of tartar emetic or stibophen.

Goodwin and Page (1943a) studied the excretion of antimony in animals and man by the polarographic method, using the following tervalent compounds, potassium antimonyl tartrate, stibophen and anthiomaline ; among the quinquevalent compounds investigated were sodium antimony gluconate (solustibosan, pentostam), neostam, neostibosan, urea-stibamine and stibacetin. In mice about 30 to 40 per cent. of the antimony content of an injection of stibophen or of the quinquevalent compounds was excreted in the urine during the first or second hour after injection. During the first hour there was a high initial rate of excretion when large doses (35 mgm. Sb/kgm.) of stibophen and sodium antimony gluconate were administered, particularly by the intravenous route. A small dose (3·5 mgm. Sb/kgm.) was excreted more slowly during the first hour, but after forty-eight hours almost the same total percentage of the dose given had been excreted at both dose levels. Weese (1938) suggested that large doses of antimony flood the whole body and that the overflow is excreted rapidly, the amount of the overflow depending on the amount injected. Tartar emetic showed a fairly constant rate of excretion over the first six hours but the rate in mice was considerably greater than that reported in human beings (Brahmachari *et al.*, 1924 ; Boyd and Roy, 1929, and Hassan, 1937). The rate of excretion of the quinquevalent compounds showed that sodium antimony gluconate was most rapidly removed : then in decreasing order came stibacetin, stibamine glucoside (neostam), urea-stibamine and neostibosan. In the tervalent group stibophen was not very much slower than sodium antimony gluconate but tartar emetic was much slower and anthiomaline slower still. Intravenous injections were in every case more rapidly excreted than those given by the subcutaneous or intraperitoneal route.

Three hours after injection into mice all measurable amounts of antimony were removed from the blood stream. In human subjects given intramuscular or intravenous injections of antimony preparations, it was found that in the case of quinquevalent sodium antimony gluconate the excretion rate was very similar to that in mice, but with stibophen the rate was slower than in mice. A dose of sodium antimony gluconate calculated to contain the same amount of antimony as a dose of stibophen was far more rapidly excreted. Individual variations, however, were marked. Khalil (1936a and b) believed that by estimating the catechol fraction of stibophen (sodium antimony *bis*-pyrocatechol-3 : 5-disulphonate) in the urine it was possible to estimate the excretion of antimony. Goodwin and Page (1943b) have shown that this assumption is incorrect since the catechol fraction is excreted in the urine very much more rapidly than the antimony of stibophen, elimination of catechol being complete in the normal subject in six hours.

However, Khalil's method may be of use for the simple detection of patients with impaired kidney function.

The Toxicity of Tervalent Antimonials and Tartar Emetic

A full review of the toxicology of antimony is given by Fairhall and Hyslop (1947).

Recent investigations of the toxicity of the tervalent antimony preparations and of potassium and sodium antimony tartrates by Goodwin (1944) have confirmed the well-established fact that quinquevalent antimony compounds are, as a rule, less toxic than tervalent preparations (Bock, 1927). A direct comparison was made of the toxicity of tartar emetic, stibophen and sodium antimony gluconate containing antimony in the tervalent and quinquevalent forms. The LD 50 in mice and the limits for each compound are shown in the table.

THE TOXICITY OF SOME ORGANIC COMPOUNDS INJECTED INTRAVENOUSLY INTO MICE (Goodwin, 1944)

Substance.	Total Number of mice used.	LD 50 mgm./20gm.	% Limits (P=0·95).
Tartar emetic (*a*) (Sb^{III})	120	1·53	91–110
(*b*) ,,	80	0·93	89–112
Sodium antimony^{III} tartrate (*a*)	60	1·14	90–111
(*b*)	100	1·15	92–109
Anthiomaline	90	3·62	88–113
Stibophen (Sb^{III})	80	31·2	81–123
Sodium antimony^{III} gluconate	70	3·44	89–113
Tartar emetic (Sb^{V})	60	5·14	96–104
Stibophen (Sb^{V})	70	66·6	88–113
Sodium antimony^{V} gluconate	80	33·0	93–108

The LD 50 values for potassium and sodium antimony tartrates are greater than those reported by Fargher and Gray (1921). Some samples of potassium antimony tartrate may be more toxic than sodium antimony tartrate or *vice versa*, thus explaining the divergence of Rogers (1918) and Fargher and Gray (1921), who found the potassium salt to be more toxic than the sodium, whereas Brahmachari (1922) found the two compounds equally toxic. Bradley and Frederick (1941) reported that after intraperitoneal injection into rats the LD 50 for potassium antimony tartrate in mgm. per 100 gm. of body weight was 3·0, corresponding to 1·1 mgm. of metallic antimony. Both the tervalent potassium salt and the quinquevalent sodium antimony tartrate are slightly less toxic after autoclaving, but prolonged boiling of tartar emetic, on the other hand, renders the solution opalescent and toxic, as a result either of dissociation or of the formation of oxides. McKenzie (1932) recorded eleven cases of acute poisoning with four deaths following intravenous injection of tartar emetic which had been boiled.

The reactions which follow the injection of tervalent preparations such as stibophen or anthiomaline are, as a rule, negligible, though in a few patients nausea and vomiting, cough or joint pains may occur, and in the latter part of the course there may be vague pains over the liver and a feeling of fatigue.

The irritant action of the tervalent antimonials, as shown by intracutaneous injection of guinea-pigs (Goodwin, 1944), is as follows :—

Compound.	Minimal necrosing concentration %.
Tartar emetic	0·1
Sodium antimony^{III} tartrate	0·2
Sodium antimony^{V} tartrate	12·5
Anthiomaline	1·0
Stibophen	12·5
Stibophen (Sb^{V})	>50

Sodium antimony tartrate is slightly less irritant than tartar emetic, while sodium antimony^{V} tartrate exhibits a greatly reduced irritant action. Stibophen containing tervalent antimony is far less irritant than tartar emetic, and stibophen with quinquevalent antimony is less irritant still.

In man the main symptoms following intravenous injection of tartar emetic are cough and a feeling of tightness in the chest, nausea and vomiting, pains over the liver, fever and giddiness with collapse and sudden death. The last complication is seen in about 0·2 per cent. of cases. Recovery may sometimes follow the intracardiac injection of 0·2 ml. of 1 in 1,000 adrenalin (Fakhry, 1931). In Egypt, according to Khalil (1935), about 2,000 deaths are seen annually from antimony poisoning. Among approximately 5,000 West Africans given tartar emetic during the war five deaths occurred.

More remote complications of tartar emetic therapy are labial herpes and a papular dermatitis. Ritchken and Kantor (1947), during the intensive treatment of patients with tartar emetic for schistosomiasis, when 13 mgm. per kilo of body weight is given in twenty-four hours, noted five cases of zoster among 105 treated. The zoster developed from five to ten days after cessation of treatment. In a series of 200 cases treated with the usual doses there was but one case of zoster. Contra-indications to the use of tartar emetic, apart from the risk of fatal accidents, are nephritis and heart failure.

The effects of tartar emetic on the heart were studied by Mainzer and Krause (1940), who found electrocardiographic abnormalities in eight of twelve patients receiving intravenous tartar emetic. The effects of stibophen on the heart have been investigated by Beaser and Rodriguez-Molina (1946). Among twenty-five patients receiving stibophen for schistosomiasis twenty showed a decrease in voltage of the T waves, coming on in fifteen patients after the third injection. The changes were, however, reversible, disappearing in three weeks or so after cessation of treatment. The ventricular gradient in one case gave a shift in direction, a point of some interest since Magalhâes and Dias (1944) suggest that the toxic effects of antimony are due to dilatation of the capillaries of the coronary system with resulting diminution in the effective cardiac circulation. Tarr (1946, 1947) published very similar results: whereas from twenty-seven to thirty-eight of forty-eight patients receiving intramuscular stibophen exhibited significant electrocardiographic changes, varying according to the dose, all of ninety patients given intravenous tartar emetic showed decrease in the voltage of the T waves. These changes were of a reversible nature and disappeared within thirty to sixty days after treatment was completed in 90 per cent. of the patients. Schroeder *et al.* (1946) analysed electrocardiograms from 100 patients at various stages of treatment of schistosomiasis with either tartar emetic or stibophen. An increased amplitude of P waves in leads 2 and 3 was found in 11 per cent. of patients, and a fusion of S and T waves in 45 per cent. of patients. Varying degrees of decrease in amplitude of T waves in all leads, resulting in a deep inversion in many instances, were noticeable in all but one of the patients, being more pronounced with tartar emetic than with stibophen. In 27 per cent. of cases the Q—T interval was prolonged beyond the limits of normal. The duration of these changes is variable; they were noted up to two months after cessation of treatment. It is not without interest that changes similar to those produced by stibophen have been seen in patients with phosphorus poisoning who die suddenly with histologically demonstrable myocardial changes. From the practical point of view these findings emphasise the fact that frequently repeated courses of stibophen or of other antimonials should be spaced four weeks or more apart so as to avoid a cumulative effect on the myocardium.

It may be noted that a quinquevalent antimonial such as ureastibamine is said to have no immediate effect upon the electrocardiogram of the dog, though it may cause weakening of the heart beat with cardiac dilatation both in dogs (David *et al.*, 1934) and frogs (Yoshimura, 1940). Schroeder *et al.* (1946) found that in patients treated with neostibosan or stibanose there were either no changes whatsoever in the electrocardiogram, or changes similar, but in a much less marked degree, to those produced by tartar emetic or tervalent antimony preparations. The main pathological lesions caused by toxic doses of antimony are found in the liver and kidney rather than in the heart (Franz, 1937; Bradley and Frederick, 1941), but from the high concentrations in the thyroid it has been suggested that toxicity may be due in part to interference with thyroid secretion.

In patients dying after administration of tartar emetic in West Africa there was extensive hepatic necrosis. Lippincott *et al.* (1947b) reported that after tartar emetic or stibophen. bromsulfalein tests and serum bilirubin determinations showed abnormal readings in a high percentage of cases: these abnormal results decreased ninety days after the end of treatment.

In the past the chief method of avoiding the toxic effects of antimony compounds has been the use of freshly prepared antimony solutions, an insistence on double glass distilled water for making up the solution, and a very slow rate of injection. The metallic taste in the mouth can be overcome by sucking chewing gum. The toxic action of stibosan can be counteracted in rats by administration of *p*-aminobenzoic acid (Sandground, 1943). Further investigations by Braun *et al.* (1946), Gammill *et al.* (1947)

and Eagle *et al.* (1947) have shown that injections of BAL (*cf.* p. 88) are capable of saving a considerable proportion of animals given poisonous doses of antimonial compounds. Thus it was found that by a single intravenous injection, in rabbits the lethal (LD 95) doses of stibophen, tartar emetic, anthiomaline and *p*-methyl-phenylstibonic acid were 150, 15, 12 and 10 mgm. per kgm. of body weight respectively. Injections of BAL at the rate of 10 to 15 mgm. per kgm., administered four times at intervals of four hours, saved approximately half the animals. This effect is probably associated with the fact that, as in the case of arsenicals, the injections of BAL increases the excretion of tartar emetic, stibophen and anthiomaline from two to eight times; the excretion of the quinquevalent *p*-methyl-phenylstibonic acid was not increased. The need for repeated injections of BAL at approximately four-hour intervals is emphasised by the fact that the favourable effect on excretion lasts only for from two to four hours.

Pak (1946) has found that by correct spacing of the doses in mice and rats it is possible to produce tolerance to all types of antimony compounds; if, however, the interval between the initial doses is too short sensitivity may result.

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SCHISTOSOMIASIS

The number of persons suffering from schistosomiasis is probably second only to that of those infected with hookworms, for schistosomiasis is found not only throughout the greater part of Africa but in China, the Philippines, Formosa, and in one of the

Japanese islands, as well as in the northern parts of South America and in many islands of the West Indies : some areas in the south of Portugal are also infected. In Egypt alone it is estimated that of a total population of fifteen million at least ten million are infected with schistosomiasis. Investigations made during the war of 1939–45 showed that in Northern Nigeria 50 per cent., and in the Gold Coast 25 per cent., of adult males from eighteen to forty years of age were infected.

In attempts to cure schistosomiasis, innumerable drugs have been used, ranging from those recommended in the Ebers papyrus to synthetic chemicals such as arsphenamine. Until the introduction of tartar emetic and emetine, time seemed the only remedy.

During the war of 1939–45 the infection of American soldiers with schistosomiasis japonica stimulated further investigations on treatment with antimony and other preparations.

Compounds of a new type, the miracils, are now under investigation.

Antimony Preparations

McDonagh (1915), in his treatise on venereal diseases, was the first to recommend the use of antimony in the treatment of vesical bilharziasis, but his patients do not appear to have been observed for a sufficient length of time to establish the permanency of the cure. Christopherson (1918), however, in the Sudan, while injecting a patient with tartar emetic for the treatment of kala azar noticed an improvement in the symptoms of bilharziasis and thus independently rediscovered the curative action of antimony, thereafter successfully treating a series of cases due to *Schistosoma hæmatobium* at the Civil Hospital in Khartoum. As a result of the publication of Christopherson's paper, McDonagh (1918) directed attention to the fact that he had treated twenty-three cases of bilharziasis with tartar emetic, colloidal antimony and "antiluetin," a double salt of potassium antimonyl tartrate and ammonium tartrate.

Owing to the toxicity of tartar emetic, other antimony compounds have been introduced, notably lithium antimonyl thiomalate (anthiomaline) and a tervalent antimony compound sodium antimony *bis*-pyrocatechol-3 : 5-disulphonate (stibophen, fouadin), originally prepared by Schmidt (1930). When first introduced it was believed that these compounds were not only far less toxic than tartar emetic or sodium antimonyl tartrate but also more efficient. The first injection of stibophen is usually 1·5 ml., the second 3·5 ml. and the third and following injections 5 ml. Results have become progressively worse. Thus Khalil (1935) in three years in Egypt obtained the following figures :—

Year.	Number of cases.	Percentage cured by			Percentage relapsed after one month.
		9 injections.	11 injections.	13 injections.	
1931	3,288	52·26	67·95	71·26	0·71
1932	3,299	62·20	82·70	86·80	0·30
1933	3,302	63·41	78·64	83·02	12·92

In 1936 Khalil reported that of 1,938 cases 33 per cent. relapsed. Cawston (1937), in South Africa, found stibophen less efficient than tartar emetic. This was also the finding in West Africa, where during the war it became necessary to treat 5,000 Africans and 168 Europeans for recently acquired bilharziasis. Despite the fact that patients received antimony within a day or two of their first developing symptoms, 86 per cent. of those receiving 40 to 60 ml. of stibophen relapsed, while among those receiving tartar emetic the relapse rate was under 10 per cent. On the other hand, there were no toxic reactions among those treated with stibophen, but among 5,000 Africans receiving tartar emetic there were five deaths attributable to the drug. In Egypt, according to Khalil (1935), there are usually about 2,000 deaths per annum from antimony poisoning. Similarly poor results in treatment were also obtained during the war with anthiomaline, the relapse rate after one course being between 70 to 80 per cent.

In Puerto Rico, Hernández-Morales, Pratt and Oliver-González (1946) came to similar conclusions as to the relative value of stibophen and tartar emetic in treatment of infections due to *S. mansoni.* Of 304 patients treated with stibophen, 202 were given one course, 62 two, and 36 three courses : 137 still showed eggs. While a single course brought about 60·4 per cent.

of apparent cures, second and third courses produced cures in only 48·5 and 36·2 per cent. Tartar emetic (6 per cent. solution) produced 36·3 per cent. of apparent cures when 60 ml. was given, 68·1 per cent. when 120 ml. was given, and a further 120 ml. increased the percentage of cures to 76·3 per cent. Tartar emetic, however, was far more toxic and entailed a longer period of treatment.

In an attempt to shorten the period of time required to treat cases of bilharziasis, Alves (1945) and Alves and Blair (1946) have studied the effects of an intensive antimony course similar to the intensive arsenical treatment used in syphilis and trypanosomiasis (p. 366). Continuous intravenous injections of 100 ml. of a 1 per cent. solution of sodium antimonyl tartrate in 5 per cent. glucose saline caused excruciating phlebalgia and were therefore abandoned. The technique of "multiple syringe injections," however, was then adopted; intravenous injections were given three times daily at three-hour intervals for two days, a total of six injections being given at 9 a.m., 12 noon and 3 p.m. on each of two consecutive days. The amount of sodium antimonyl tartrate injected at each dose was from 7 to 14 gr. and, being based on body weight, roughly 1 gr. of sodium antimonyl tartrate being given for every 12 lb. of body weight. Thus a man of 144 lb. body weight would get a total dosage of 12 gr. of sodium antimonyl tartrate. Examples of the dose schedule of sodium antimonyl tartrate are as follows :—

	9 a.m.	12 noon.	3 p.m.	9 a.m.	12 noon.	3 p.m.
Patient A, 10 gr. (0·6 gm.) given as	2	2	1	2	2	1
Patient B, 13·5 gr. (0·81 gm.) given as	2	2	2·5	2·5	2·5	2
Patient C, 9·25 gr. (0·55 gm.) given as	2	2	1	2	1·25	1

Each injection was given in 10 ml. of 5 per cent. glucose saline into a vein in the antecubital fossa at a rate not exceeding 2 ml. per minute. The slow and steady injection of the drug is considered to be of fundamental importance in the success of the treatment. Among 100 patients toxic reactions were in no case sufficient to lead to the interruption or discontinuance of the course, while the more troublesome manifestations which usually accompany the intravenous injection of antimony were conspicuously absent. Cough was seen in only 16 per cent. of cases, during, or more commonly after, the first injection : it never lasted for more than two minutes, and the frequency of its occurrence diminished throughout the course : only two patients suffered from it as a result of their sixth and final injection. Vomiting was not seen in association with a first injection, but was encountered in six in association with the second, and in two in association with the third injection ; the same symptom was noted in three cases, and in one respectively after the fifth and sixth injections. A feeling of constriction of the chest was noted in only one patient, an asthmatic ; abdominal cramps were seen in three patients. There was no instance of collapse and no rheumatic pains, but salivation was common after the earlier injections though it had usually vanished by the second day. Two patients had considerable hæmaturia after the third injection, but in neither did it persist. The average rate and regularity of the pulse were not altered by the injections. Urticaria and zoster have been seen in West and South Africa in association with the injection of antimonyl tartrates. Ritchken and Cantor (1947) observed five patients with zoster after intensive antimony therapy. A very considerable degree of euphoria was noted both in Africans and in Europeans who had completed the course.

Both for diagnosis and for gauging the results of treatment, reliance was placed largely on the intradermal use of a cercarial antigen. Of fifty-three cases originally positive before treatment only fourteen gave a positive skin test two months after the end of treatment. Nine of the fourteen positive cases were re-tested one month later, six had by then become negative, two remained positive, and one, originally strongly positive, was only weakly positive. In no case examined two months after treatment were eggs found in the excreta. Studies on the urinary excretion of antimony during this intensive course showed that 70 to 80 per cent. of the antimony injected remains in the body seventy-two hours after the beginning of the treatment. The high antimony concentration thus attained may be responsible for the rapid disappearance of the ova in these cases.

A one-day treatment of schistosomiasis has also been developed by Alves (1946): 131 persons were divided into three groups and given the following treatment:—

Group A received 6 gr. (0·36 gm.) of sodium antimonyl tartrate in three doses of 2 gr. (0·12 gm.) at four-hourly intervals.

Group B received 7·5 gr. (0·45 gm.) of sodium antimonyl tartrate in three doses of 2·5 gr. (0·15 gm.) at four-hourly intervals.

Group C received 8 gr. (0·48 gm.) of sodium antimonyl tartrate in four doses of 2 gr. (0·12 gm.) at three-hourly intervals.

These injections were unaccompanied by any severe reactions.

Alves and Blair (1947) believe that it is essential to give 1 gr. (0·06 gm.) of sodium antimonyl tartrate for every 12 lb. of body weight. One injector can thus deal with twenty-four patients in a day.

It must be noted, however, that the negro does not stand up well to the toxic effects of tartar emetic, and necrosis of the liver with death is not uncommon. Alves and Blair (1947) reported four deaths in Africans in association with intensive antimony treatment.

In another series of Africans they noted severe collapse in a small boy when only 6 ml. of the drug had been injected. Patients were examined two and four weeks later: at the first examination seven were passing a few dead eggs, while at the second one was passing dead eggs. In no instance were live eggs found. Mills (1946) has attempted to intensify the course of anthiomaline or stibophen by giving daily intramuscular injections of one or other drug for six consecutive days during each of two successive weeks. Apparent cure occurred in forty-two of forty-six West African soldiers examined up to three months after termination of the course. Further examination of some of these patients up to six months after treatment showed that they had either relapsed or been reinfected. Toxic complications with either anthiomaline or stibophen were slight, but paresis of the facial muscles continued in some cases for about a week.

Talaat and Shoaib (1947), in Egypt, have investigated a number of treatment schedules in which tartar emetic is given in large amounts for a short period. The treatment by three intravenous injections three hours apart on each of two consecutive days was found to produce profound circulatory collapse after the third or fourth injection. Similarly, the one-day course of three intravenous injections produced such severe reactions in Egyptians that it had to be abandoned. The safest course appeared to consist of two injections of 0·12 gm. each in 10 ml. of saline given two hours apart for two consecutive days.

Halawani and Abdallah (1946) treated fifteen Egyptians with "reprodal," which is said to be identical with stibophen. Six injections of 5 ml. were given at three-hourly intervals on two successive days, thus giving 255 mgm. for a 60-kgm. adult. All the patients had infections due to *S. hæmatobium*. Of the fifteen treated only eleven are said to have been cured. No changes in the electrocardiograms of these patients were noted by Hammouda (1946), who also treated with success two patients aged twelve and fifteen years.

Talaat and Shoaib (1948) similarly treated ninety patients, 0·12 gm. of tartar emetic being given as before on two consecutive days. Patients were followed for from one to seven months: seventy-three were free from ova, two passed dead ova and fifteen were still passing living ova. Children under ten years received one additional injection on the third day. Provided urinary output is good, renal disease is not a contra-indication nor is anæmia, but cyanosis and decompensated heart lesions are.

More intensive methods of treating schistosomiasis are obviously of considerable importance but prolonged examination up to at least six months is essential to make certain that cure is permanent, for oviposition may be temporarily inhibited without killing the worms. Periodic examinations should involve cystoscopic or sigmoidoscopic examinations. The injection of cercarial antigen to test for skin sensitivity is of doubtful value in estimating cure. In addition, if schistosomiasis is to be eliminated, chemotherapeutic treatment must be combined with snail destruction for reinfections are only too common in areas where the infestation rate of snails is high.

The use of quinquevalent antimonials in the treatment of schistosomiasis has not as a rule met with any great success. In fact, on somewhat doubtful grounds, the failure of stibophen has on occasion been blamed on its oxidation to quinquevalent antimony. Hernández-Morales *et al.* (1946a) have, however, employed urea stibamine intravenously in the treatment of fourteen cases of infection with *Schistosoma mansoni.* The total amount of the drug tolerated varied from 3·4 to 10·1 gm., given over a period of from thirteen to eighteen days. At the end of treatment three patients were no longer passing eggs either dead or alive, and one other patient became negative thirty days after the end of treatment. Of the remaining ten patients one died on the ninth day of treatment after receiving 5·03 gm., but of the others seven showed reduction in the number of eggs passed in the fæces. Immediate toxic reactions were common, facial œdema, hoarseness, tachycardia, abdominal pain, nausea, vomiting, sweating, bradycardia, pallor and a shocked condition. Four patients continued negative for a period of twelve months, but of twelve patients treated with neostibosan only four were free from ova eleven months later. Stibanose was useless (Hernández-Morales *et al.*, 1946b).

In the hands of Hernández-Morales and his colleagues (1946c) anthiomaline has given good results in infections due to *S. mansoni:* in all forty patients were treated, thirty-three of these received 3 ml. of the solution, equivalent to 30 mgm. of antimony, intramuscularly every other day, but treatment had to be discontinued after 12, 24, 28 and 36 ml. had been given in four patients, because of bradycardia, joint pains, fever, epigastric discomfort, anorexia and loss in weight. The remainder received a total dosage of 45 ml. Of twenty-four of these patients followed up for from three to nine months, only one showed a recurrence of infection. Seven patients were given daily doses of 3 ml. but only three were able to complete the course of 45 ml. The stools of all were free from ova one to three months later. A progressive eosinophilia without any significant alteration in the red cell count was common and appears to be a result of the destruction of the parasitic worms. It has been noted also in urinary schistosomiasis.

Oliver-González and Hernández-Morales (1946) have shown that in patients treated with anthiomaline and urea stibamine living and dead eggs disappear from the fæces at the same time : dead eggs do not persist.

Gonzalez Rincones (1945) has devised a method of administering tartar emetic by mouth, in the form of pastilles. Good results it is claimed are obtained in the case of infections due to *S. mansoni*. Every centigram of tartar emetic is combined with 0·05 mgm. of atropine and a small amount of aneurin. A new tervalent antimony preparation has been introduced by Tavares da Silva (1947) for the treatment of infections due to *S. mansoni*. This compound termed Stibophen III is said to contain 8 per cent. of metallic antimony and 30 per cent. of iodine : it is described as hexaiodomethoxyquinoline-hydroxymethylquinuclidine antimoniate. Its superiority over other antimony derivatives is not apparent from the results so far obtained.

A possible new approach to the chemotherapy of schistosomiasis has been indicated by Brandt (1947), who has found that in the case of *S. mansoni* infections in rabbits and monkeys the injection of heparin localises the worms in the portal vein and liver. It is suggested that when this has been done anthelminthics might be introduced in high concentration directly into the portal vein. In the earliest stages of infections due to *S. mansoni*. in mice antimonials gave from 40 to 100 per cent. protection (Schubert, 1948). It is possible to protect mice against infection with *S. mansoni* by adding rosaniline base (CI 677) to the diet to the extent of 0·33 per cent of the food. Where this was done for 7 days before infection and 28 days after infection 90 per cent of mice where protected, whereas only 13 per cent of control mice were not infected (Wright *et al.* 1948).

Treatment of Japanese Schistosomiasis

Stibophen has been used in the treatment of infections due to *Schistosoma japonicum* by Lee and Chung (1933). Li and Thompson (1934) and Kan and Kung (1936), all of whom have noted temporary improvement followed by a tendency to relapse, the percentage of permanent cures being less than with tartar emetic. Mason *et al.* (1946) found that of 100 American soldiers treated with stibophen eighteen were cured, whereas of the same number treated with tartar emetic eighty-one were cured. Apart from transient cough, nausea and slight shoulder pains in three-quarters of the patients there were no toxic reactions. The T wave of the electrocardiogram was diminished in all those who received tartar emetic and in half those treated with stibophen, but no clinical evidence of heart damage was found. Liver function tests showed a transient impairment. Billings *et al.* (1946) treated 110 cases, all recently infected in the Pacific area, with 40 ml. of stibophen : nineteen had mature viable ova in the fæces at the end of the course, though in all cases fever had ended four weeks after the beginning of treatment. Lippincott *et al.* (1947) found that of thirty-three patients infected with *S. japonicum* and given a full course of stibophen, twenty-seven were passing eggs within three months of cessation of treatment. These twenty-seven were given a second course and within three months twenty-four were again passing viable eggs. Of fifty-nine patients given tartar emetic forty-eight were cured. It is thus obvious that stibophen is not of great value for the permanent cure of Japanese schistosomiasis.

The Effects of Antimony in Experimental Schistosomiasis

The mode of action of antimony compounds on schistosomes has received considerable attention. Nishi (1923) demonstrated that in dogs infected with *S. japonicum* tartar emetic caused degeneration of the ovaries and loss of hæmatin in the adult worms. Fairley (1926–27) confirmed these results in goats infected with *S. spindale.* Giovannola (1936) showed that stibophen was less active than potassium antimonyl tartrate in killing adult *S. mansoni* worms in rabbits, and Lee (1932), using the same animals, found that though the ova of *S. japonicum* might temporarily disappear from the urine as a result of the administration of stibophen, there were many relapses. Stibophen injected intravenously was less effective than when given intramuscularly. Khaw (1934) employed a " concentrated fouadin " experimentally in rabbits, 1 ml. of the concentrated preparation, a pyrocatechol disulphonate of calcium and sodium, containing 14·3 mgm. of tervalent antimony instead of 8·5 mgm. When given intramuscularly to rabbits infected with *S. japonicum*, fourteen were cured, five were killed and one was still infected: in those that died the livers showed much fatty change.

Vogel and Minning (1947) find that the action of tartar emetic, stibophen and emetine on *S. japonicum* is very similar and is limited to the testes, ovaries and yolk glands, which first show a relative increase in the number of ripe cells, quickly followed by a considerable diminution.

More extensive observations have been made by Bang and Hairston (1946) on the effects of stibophen, anthiomaline and tartar emetic on guinea-pigs and dogs infected with *S. japonicum.* Making use of the information obtained by Vogel (1942) that the development of the egg takes about three weeks, it was possible to show that these drugs have no direct action on the egg but only on the adult worm. The first effect on the worms was to cause them to migrate from their normal position in the branches of the mesenteric veins near the intestine into the portal vein either near its entrance to the liver or in the smaller branches of the portal vein within the liver. After two weeks of treatment with stibophen the worms were shrunken, most of the hæmatin had disappeared from the intestine, the ovaries had lost their cellular structure and contained brown granules, the vitelline gland was largely destroyed, and eggs were few in number or entirely absent.

Tartar emetic was the most effective drug; stibophen was less active than anthiomaline. Neostam, in comparable doses by weight of antimony, had little or no effect. Schubert (1948a and b) tested more than 400 drugs in mice infected with *S. mansoni.* Those which were effective were divisible into three groups:—

(i) Drugs which left an average of 0 to 1 worm per mouse.
(ii) Drugs which produced a 50 per cent. reduction.
(iii) Drugs of slight or doubtful value.

Group I included antimony tri (2, naphthyl-ethylmercaptide); antimony tri (*n*-dodecylmercaptide); *n*-butyl 3, 4 antimonyl gallate.

Group II included stibophen, antimony trithioglycollamide; antimonyl 2 mercapto-thiazoline; antimony tri (2, mercaptoiminazoline); antimonyl tri (*n*-decylmercaptide); neostibosan, neostam, phenyl mercuric gluconate; emetine hydrochloride and miracil D.

Group III included sodium antimonyl tartrate, benzylammonium antimony tartrate, sodium potassium tartroantimonyl caffeate, sodium antimony catechol tartrate, trihydroxymethylaminomethane antimony catechol, *n*-butyl 3, 4 antimonyl gallate, methyl 3, 4 antimonyl gallate, sodium antimony catechol thioglycollate, antimony trithioglycollamide, antimony tricysteine, antimony tri (*n*-dodecylmercaptide), antimony tri (*n*-tetradecylmercaptide), antimony tri (*n*-octadecylmercaptide), phenyl antimony dicysteine, and solustibosan.

The effective drugs thus include both ter- and quinquevalent antimonials. It is a point of some interest that among the ten antimonials of the first two groups five are of the type $Sb\,(SR)_3$ and, of these, four are fat soluble rather than water soluble. Oil-soluble antimonials were prepared by Clemence and Leffler (1948).

Bieter *et al.* (1949) find that 6-methoxy-8-β-di*iso*butylaminoethylamino quinoline dihydrochloride and the *iso*propylaminoquinoline are effective against *S. mansoni* in mice.

Naphthoquinones and Schistosomiasis

According to the observations of Bueding *et al.* (1947), 2-methyl-1, 4-naphthoquinone inhibits aerobic glycolysis of *S. mansoni in vitro.* Since glycolysis rather than oxidation appears to be essential for the survival of these organisms, and since the toxicity of 2-methyl-1, 4-naphthoquinone is low for mammalian species, experiments were carried out in which infected mice were fed on a diet containing 1 to 1·5 per cent. of this compound and at the same time were given subcurative doses of stibophen, 7·5 mgm./kgm. every eight hours for five days. The schistosomes removed from mice receiving both drugs together showed a much lower rate of glucose consumption and lactate formation than did those from mice receiving stibophen alone. It is possible that 2-methyl-1, 4-naphthoquinone acts synergically with subcurative doses of antimonials. 1, 2-Naphthoquinone is a far less potent inhibitor of glycolysis, and the same is true of 3-methoxy-2 methyl-1, 4-naphthoquinone and substituted 3-OH-naphthoquinones.

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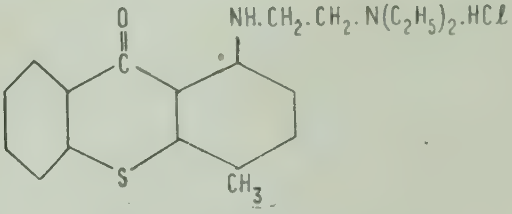
MIRACILS

During the war years an entirely new class of compound was synthesised in Germany. One of these compounds, 1-methyl-4-β-diethylaminoethylaminothioxanthone hydrochloride, miracil D, is active against schistosomiasis in animals. In mice infected with *Schistosoma hœmatobium* the chemotherapeutic index is 1 : 4. It appears to be from five to ten times as active as antimony in *S. mansoni* infections in mice, guinea-pigs and monkeys. In monkeys a single dose of 20 mgm. per kgm. of body weight by mouth in two equally divided doses produces a cure. Adult worms are more susceptible than immature forms, Kikuth *et al* (1946).



Miracil D (Nilodin, RP 3,735).

Bueding *et al.* (1947) confirmed the therapeutic effect in mice : eighteen doses every eight hours of 36 mgm. per kgm. given intraperitoneally killed all adult worms in half the mice, but only worms in the liver. A quarter of the mice died from the drug. The surviving flukes showed a decreased rate of glycolysis. When added to living flukes *in vitro*, miracil did not inhibit either respiration or glycolysis nor did any compound which could be so obtained from the tissues. The hydrochloride of miracil has a melting point of from 195°–196° C. The pharmacology of the drug has been studied by Wood (1947). In mice, single doses of 1 gm. per kgm. of body weight or ten daily doses of 125 mgm. per kgm. of body weight were tolerated by mouth. Rabbits tolerate single oral doses of 150 mgm. per kgm., but are killed by four such doses. Intravenously in mice, the LD 50 is 45 mgm. per kgm. ; in rabbits, by the same route, doses of 20 mgm. per kgm. were tolerated, but four such doses produced convulsions within three minutes, the convulsions resembling those due to picrotoxin. Some animals showed head retraction with extended limbs. Histological examination of rats and a rabbit which had died from miracil D did not reveal any definite changes. Repeated daily oral doses of 30 mgm. per kgm. retarded the growth of rats, but daily oral doses of 20 mgm. per kgm. of body weight had no appreciable effect on growth, hæmoglobin concentration in the blood or total leucocyte counts. When given intravenously in anæsthetised cats or rabbits, doses larger than 5 mgm. caused a fall in blood pressure in part due to direct action on the heart, in part to peripheral vascular dilatation. There was no significant action on respiration, spleen volume or on the rat phrenic-nerve-diaphragm preparation with doses of 1 to 10 mgm.

Further studies on the pharmacology and toxicity of miracil D have been made by Halawani *et al.* (1947), Hawking and Ross (1948), Kikuth and Gönnert (1948) and Blair *et al.* (1949a). When applied locally miracil D is irritant to the tissues and given subcutaneously or intramuscularly it causes inflammation and some necrosis : intravenously it is more toxic than by mouth and may cause thrombosis of veins.

Hawking and Ross (1948) found that the maximum tolerated dose for mice was as follows :—

Oral . . .	300 to 1,000 mgm. per kgm.
Subcutaneous . .	340 to 500 mgm. or more per kgm.
Intravenous. . .	20 to 30 mgm. per kgm.

Repeated daily doses of 125 mgm. per kgm. could be given to mice by mouth for ten days. In mice infected with *S. mansoni* the minimum curative dose by mouth was 120 mgm. per kgm. for six successive days.

Rabbits were found to tolerate a single dose of from 600 to 800 mgm. per kgm. by mouth but only 15 to 20 mgm. per kgm. intravenously : however, they tolerated twenty-eight daily oral doses of 50 mgm. per kgm. but died after twelve or fourteen daily oral doses of 100 mgm. per kgm.

Kikuth and Gönnert (1948) also found that mice could tolerate 300 mgm. per kgm. by mouth but 500 mgm. caused the death of half the animals. Rabbits after 700 to 1,000 mgm. per kgm. showed no immediate symptoms but died later. After intravenous injection into rabbits, as well as cats, convulsions occurred but the animals recovered : 40 mgm. per kgm. was quickly fatal. In rabbits repeated administration of 50 to 100 mgm. per kgm. by mouth or of 20 mgm. per kgm. intravenously caused lesions from which some of the animals died. Cats are particularly sensitive to miracil D and cannot tolerate daily doses of even 10 mgm. per kgm. without ill effects ; vomiting was frequent, and even a week after cessation of treatment a yellow discoloration of the aorta persisted. In rabbits, and in cats to an even greater extent, fatty degeneration was present in the liver, kidneys and heart. With doses of 400 mgm. per kgm. daily, death occurs in eight or more days. Rabbits exhibit loss of weight, hæmo-concentration and albuminuria. When 0·1 ml. of a 1 per cent. solution is dropped into the conjunctival sac of the rabbit œdematous-purulent conjunctivitis results.

Monkeys tolerate 200 mgm. per kgm. four times a week by mouth (Wood, 1947).

The pharmacology and toxicity of miracil D in monkeys has been studied by Hawking and Ross (1948) and Kikuth and Gönnert (1948). When miracil D is given by mouth absorption is rapid and the blood concentration is sustained for at least twenty-one hours after a single dose of 400 mgm. per kgm. is given by stomach tubes. Blood concentrations are not in proportion to the dosage given. Thus a single dose of 10 mgm. per kgm. gives a concentration of 0·1 mgm. per litre at two hours but none at six hours. A dose of 20 mgm. per kgm. causes a blood concentration of 0·45 mgm. per litre at 2½ hours. Miracil is excreted in the urine.

The Concentration of Miracil D and its Degradation Product in the Organs of Two Monkeys.

(Hawking and Ross, 1948)

Concentrations in mgm. per kgm.

$$\text{Ratio} = \frac{\text{Concentrations of degradation product}}{\text{Concentration of miracil}}$$

Organ.	Miracil D.		Degradation product.		Ratio.	
	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.
Brain . .	3·5	1·3	60	24	17	18
Muscle . .	30	5·4	1·8	2·2	0·006	0·41
Liver . .	25	12	80	140	3·2	12
Heart . .	21	16	6·0	9·8	0·29	0·61
Kidney . .	93	56	8·0	13	0·09	0·23
Lung . .	98	63	3·0	5·0	0·03	0·08

Monkey No. 1 had received twelve doses of 400 mgm. per kgm. for eighteen days and monkey No 2 thirteen doses of 200 mgm. per kgm. in thirty days and six doses of 400 mgm. per kgm. in nine days. In the degradation production the basic character of the molecule is masked or destroyed.

Kikuth and Gönnert (1948) found that in monkeys single doses of 400 or 800 mgm. per kgm. caused vomiting, and 400 and 200 mgm. per kgm. caused slight diarrhœa. Toxicity in monkeys is very low : slight loss of weight may occur during a therapeutic course, but this may have been due to toxins liberated from the worms. At the beginning of a therapeutic course monkeys may vomit, but this symptom disappears later. The tissues may show yellow staining, especially in the liver, but no pathological lesions.

In man miracil D is rapidly absorbed from the alimentary tract and 2¼ hours after a single dose of 0·2 gm. the concentration in the blood rises to about 1 mgm. per litre. The blood concentration is correlated with the urea clearance rate (Halawani *et al.*, 1947).

Hawking and Ross (1948) studied the behaviour of miracil D in six volunteers. The height and persistence of the concentration in the blood varies considerably in individuals. In persons taking repeated doses the blood concentrations on the second day tend to be higher than those on the first day but thereafter the blood concentration does not rise. After ceasing to take miracil D no drug concentrations could be found forty-eight to seventy-two hours later. Over 90 per cent. is degraded in the body and only about 7 per cent. is excreted in the urine. The concentration in the urine may reach 30 mgm. per litre, but is usually lower. Three days after ceasing to take the drug none can be found in the urine. Little miracil D is excreted in the fæces : with a dose of 100 mgm. daily the fæces thirty hours later contain about 2 mgm.

In Egypt, Watson *et al.* (1948) observed toxic symptoms in about 20 per cent. of patients. Anorexia, nausea, vomiting, vertigo and tremors occurred usually on the first day of treatment and then diminished in intensity. This phenomenon may be associated with a low clearance rate. Some patients appear to have an idiosyncrasy, since they suffer from restlessness, sweating, insomnia, nausea, metallic taste, tinging of the skin, headache and lumbar pain. No evidence was obtained of any involvement of the heart. In Egypt and the Anglo-Egyptian Sudan toxic symptoms have been more pronounced than in Southern Rhodesia. In the latter area toxic symptoms consisted mainly of abdominal pain, nausea, vomiting and cough, sometimes with slight disorientation. The frequency of these symptoms did not appear to be associated with the size of the dose and was related, at least partially, to personal idiosyncrasy. Blair *et al.* (1949a) saw one boy with generalised pruritus and herpes after taking 6·6 gm. in fifteen days. It would seem that such lesions, seen also with antimony, are due to toxins liberated by dying schistosomes.

Treatment of Schistosomiasis

Miracil D is an orange-yellow powder soluble in water at room temperature to the extent of 1 to 2 per cent. In treating *S. hæmatobium* infections in Egypt (Abdel Azim *et al.*, 1948 ; Watson *et al.*, 1948), various dose schedules were employed, varying from 400 mgm. twice or thrice at intervals of three days up to 300 mgm. at twelve-hourly intervals for fourteen days. With lower dosage the results were erratic. The number of viable ova in the urine and fæces decreased and usually ova disappeared for a time ; after periods of from three to eight weeks, however, they usually reappeared, though in small numbers. With larger doses some cures were obtained. Improvement appeared to be substantially less in children than in adults receiving the same dose rate per kgm. of body weight.

In Egypt it was believed that results were as good with *S. mansoni* as with *S. hæmatobium*, but so many dose schedules were used and the numbers treated with each were so small that it is difficult to draw conclusions. In Southern Rhodesia, Blair *et al.* (1947) at first obtained poor results in thirty-eight patients with infections due to *S. hæmatobium* and *S. mansoni*. Two months after treatment eighty-six per cent. of patients were still excreting ova. Later Blair *et al.* (1949b) treated 102 children with infections due to *S. hæmatobium*. The dose schedules were :—

(1) 15 mgm. per kgm. daily for five days (total 75 mgm. per kgm.).
(2) 20 ,, ,, ,, ,, (,, 100 mgm. per kgm.).
(3) 25 ,, ,, ,, ,, (,, 125 mgm. per kgm.).

The first was regarded as unsatisfactory, the second as satisfactory and the third as ideal. The results are shown in the table.

THE EFFECT OF MIRACIL D IN SCHISTOSOMIASIS.
(Blair *et al.*, 1949b)

Daily dose of miracil D for five days.

	15 mgm./kgm.				20 mgm./kgm.				25 mgm./kgm.			
Treatment. *S. hæmatobium* infections.	Cured.	Dead eggs.	Failed.	Total.	Cured.	Dead eggs.	Failed.	Total.	Cured.	Dead eggs.	Failed.	Total.
Ideal .	25	2	2	29	14	5	2	21	3	—	—	3
Satisfactory .	7	—	2	9	14	3	1	18	1	—	1	2
Unsatisfactory	5	—	5	10	5	—	2	7	—	—	3	3

The concentration of drug in the plasma is twice that in the red blood cells, but the concentration in the leucocytes is greater. In man the maximum tolerated dose was 0·2 to 0·6 gm. per day. Over-dosage caused nausea and prostration; insomnia and yellow discoloration of the skin and sclerotics occurred. Constipation is more common than diarrhœa. These symptoms come on about twenty-four hours after a dose and are apparently due to some degradation product. Of eighty-two children given ideal or satisfactory courses seventy-four ceased to pass living eggs or active miracidia. In *S. mansoni* infections results were poor, for of fifteen persons only three were cured with similar doses. The view that *S. mansoni* is more resistant to miracil D than *S. hæmatobium* is contrary to the claims made in Egypt.

Somewhat larger doses of miracil D have been used by Blair *et al.* (1949b) in the treatment of bilharziasis in Southern Rhodesia. Forty young persons with infections due to *S. hæmatobium* were given doses of approximately 80 to 100 mgm. per kgm. during periods of approximately three weeks (4 mgm. per kgm.). Twelve weeks later eleven of them were free from infection. Thirteen boys were given doses ranging from 35 to 150 mgm. per kgm. over a period of fifteen days, given irregularly as daily doses of 0·6 gm. per person. Three weeks after treatment ten of the thirteen were no longer passing ova of *S. hæmatobium*. Four months later four of the boys were apparently still cured. Eighteen school children infected with *S. hæmatobium* were given total doses of about 90 to 100 mgm. per kgm. during ten days, mostly as daily doses of 0·5 to 0·7 gm. per person (about 10 mgm. per kgm.). Six weeks later fourteen or sixteen no longer passed ova and fifteen weeks later twelve of eighteen appeared permanently cured. Doses up to 1·6 gm. daily were given to fifteen other patients, many of them adults.

Although in Southern Rhodesia the use of chocolate-coated tablets has been followed by relapses, miracil D is capable of curing a proportion of cases. Miracil D has no action on *S. japonicum* (Vogel and Minning, 1948). Halawani *et al.* (1947) have pointed out that when the kidney function is impaired the blood concentration of miracil D, and presumably its therapeutic potency, are increased.

The mode of action of miracil D has been studied especially by Kikuth and Gönnert (1948). The miracils show poor activity against immature worms both in mice and monkeys. This can be correlated with the findings in the ovaries, testes and yolk-glands, very similar to those described for antimony. Within a few days of the administration of miracil D, egg production becomes disturbed. Eggs, deformed or without shells, are produced and conglomerations of shells and yolk cells are formed while shell substance is excreted in large drops. The worms lose transparency, shrink in size, and the uniform filling of the intestine with pigment is interrupted. Worms do not die until about fourteen days after the beginning of treatment. It would seem that though miracil D does not inhibit mitosis it attacks those organs which have the most active metabolism.

Estimation of Miracil D in Urine

Coxon *et al.* (1947) devised the following method for estimating miracil D in urine. To 50 ml. of urine in a separating funnel is added a sufficient volume (2 to 3 ml.) of 0·3 N NaOH to render the mixture alkaline to litmus and also 50 ml. of diethyl ether; the funnel is vigorously shaken. After settling, the lower layer is collected in a flask and the upper transferred to a stoppered bottle. The lower layer (which is likely to contain some emulsion) is now returned to the funnel and shaken with a further 50 ml. of ether. On separation, the upper layer is added to the ether extract obtained from the first operation, while the lower is again returned to the funnel and mixed with about 100 ml. of water. This tends to break up any remaining emulsion, enabling a further amount of ether extract to be recovered. The whole of the ether extract is now washed with 10 ml. of 0·3 N NaOH, any emulsification being counteracted with a few drops of absolute ethanol. The ether layer, after a further washing with water, is then shaken with exactly 10 ml. of N HCl. The acid layer is withdrawn and its colour compared with standards made up by dissolving 5 and 10 mgm. respectively of miracil in 1 litre of N HCl.

If the reading indicates a urinary content of less than 0·5 mgm./1 of miracil, it is preferable to repeat the procedure using 100 ml., or even 200 ml., of urine.

Miracil D can be identified by means of the ruby colour which it gives when treated in chloroform solution with acetic anhydride and concentrated sulphuric acid. Urine from a rabbit injected with miracil contains in addition to miracil, identifiable by this test, other yellow material which can be extracted into ether but which cannot be transferred therefrom to HCl.

The concentration of the drug in rabbit urine following a dose of 25 mgm./kgm. body weight was about 2·5 mgm./1.

Estimation of Miracil D in Blood

Two methods for the estimation of miracil in blood have been suggested by Coxon *et al.* (1947) and Latner *et al.* (1947): the dye-laking method and the yellow colour method. The dye-laking method consists in extracting miracil from laked blood in the presence of sodium phosphate into ethylene dichloride. To this is added bromothymol blue (buffered at *p*H 7) and the mixture is shaken with the formation of a compound of miracil and bromothymol blue which is soluble in ethylene dichloride. Since the uncoupled dye is insoluble in the organic solvent, a partition of the dye takes place and the amount of the dye thus removed from the aqueous phase is measured.

(i) The actual technique of the dye-laking method is as follows :—

Bromothymol blue reagent: This is prepared in two stages as follows :—

First a stock solution is made by dissolving 40 mgm. of solid dye in 100 ml. of 1 per cent. alcohol; secondly, 4 ml. of this solution are diluted to 100 ml. with buffer containing 3·63 gm. of KH_2PO_4 and 14·35 gm. of $Na_2HPO_4 . 12H_2O$ to the litre.

To 5 ml. of fresh whole blood, laked with 10 ml. of water, are added 3 ml. of 0·2 m-Na_2HPO_4, and then 30 ml. of ethylene dichloride. The mixture is shaken vigorously for five minutes in a mechanical shaker, and then centrifuged for fifteen minutes at 2,500 revolutions per minute. The upper aqueous layer is aspirated away, leaving the organic layer covered with a pellicle of protein material.

The ethylene dichloride is transferred to another bottle, the pellicle being left behind, and is then washed once with 60 ml. of 10 per cent. NaOH and twice with the same volume of water. At each stage the mixtures are mechanically shaken and later separated by centrifuging as in the original extraction. 25 ml. of the ethylene dichloride layer are removed by means of a teated pipette, and placed in a small stoppered measuring cylinder; 2·5 ml. of bromothymol blue reagent are then added and the mixture is shaken mechanically for fifteen minutes to bring about coupling between miracil and the dye, and is then centrifuged.

One ml. of the upper aqueous layer is removed and treated with a micro-drop of 10 . N NaOH. The resulting blue colour is read in a Spekker absorptiometer, using microcups, and the original miracil content is determined from a graph previously prepared. The graph is drawn from the readings obtained, after treating by the above method a series of samples of normal fresh blood to which miracil has been added in known amounts.

(ii) The yellow colour method. Miracil exhibits a strong yellow colour when dissolved in hydrochloric acid, this colour varying with the concentration of acid present. By transferring miracil from 5 ml. of blood into 1 ml. of 0·04 N HCl and reading the colour of the latter in a Spekker absorptiometer using microcups, blood concentrations of the order of 10 to 100μg. per 100 ml. can be handled.

Calibration of Instrument. A stock standard solution containing 100 mgm. miracil hydrochloride in 1 litre 0·1 N-HCl is prepared. This is stable for at least three months, if kept in the dark. Two ml. of this solution are diluted to 50 ml. with distilled water. Each 0·5 ml. of this dilute solution, when added to 5 ml. of fresh oxalated blood, produces a miracil content equivalent to 40 μg./100 ml. A series of 5 ml. blood samples can, therefore, easily be prepared containing total amounts of miracil hydrochloride equivalent to concentrations of 40, 80, 120, 160 and 20 μg./100 ml. respectively.

Each sample is hæmolysed with 10 ml. distilled water, and then made alkaline with 2·5 ml. N-NaOH solution. It is best to carry out the whole of this procedure, from the preparation of the 5 ml. blood sample onwards, in a 500-ml. separating funnel. The clear brown solution thus formed is treated with 0·1 ml. caprylic alcohol, and thoroughly extracted by shaking with 25 ml. of ether. It is essential to shake the separating funnel at least 200 times. The emulsion thus formed is, as a rule, easily broken by the addition of 2 ml. of acetone and is allowed to stand for at least half an hour until the two layers are well defined. If the emulsion proves especially obstinate, a few drops of absolute alcohol may be added as a further aid to inducing adequate separation. After the lower layer has been run off, the ether layer is collected and set aside in a stoppered bottle while the lower layer is returned to the funnel and again extracted with a further 25 ml. ether. Separation at this stage usually takes place quite easily. Occasionally, however, it may be necessary to add a few drops of absolute alcohol. The ethereal phase from the second shaking is added to that obtained from the first, the residual aqueous fluid being discarded. The separating funnel is now washed out with 20 ml. 0·5 N-NaOH solution to remove any scum adhering to inner surface, and the combined ether extracts are returned to the cleaned funnel, and shaken well with a further 20 ml. 0·5 N-NaOH. The alkali layer is run off, and the ether extract washed twice by shaking well with distilled water whose reaction has been adjusted to *p*H 7·2. Each of these procedures is liable to be followed by some degree of emulsion formation, but standing for ten minutes is usually sufficient to bring about an adequate degree of separation. It has been demonstrated that the small amount of ether contained in the emulsions, after this period of time, is usually negligible, and its loss does not materially affect the result.

The ether extract, after the final washing, is freed as far as possible from the lower aqueous layer, and transferred to a clean dry 100-ml. separating funnel. This transfer is carried out by pouring the ether through the neck of the larger funnel, and not by running it off through the stem, so as to avoid carrying over any traces of emulsion which might dilute the acid to be added. One ml. of 0·04 N-HCl is now introduced and the small funnel thoroughly shaken by hand for several minutes. There must be at least 600 to-and-fro movements.

After being allowed to settle, the clear lower layer, which is now, as a rule, perceptibly yellow in colour, is run off into a microcup (volume 0·5 ml.) and its coloration measured in a Spekker photoelectric absorptiometer, using an ultra-violet light source, and spectral violet filters (Ilford 601).

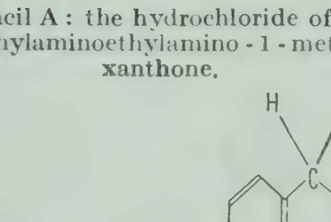
Determination of Miracil Concentrations in Blood. Five ml. of oxalated blood are treated as already described, and the miracil content determined (in terms of miracil hydrochloride, μgm./100 ml.) from the calibration curve.

Miracils A, B and C

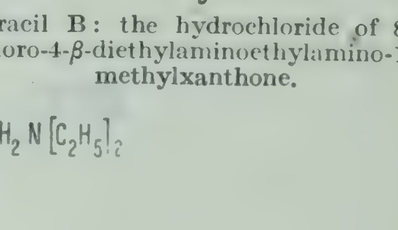
In addition to miracil D, which is a thioxanthone, Mauss (Kikuth and Gönnert, 1948) synthesised certain other closely allied compounds. Miracil A and B are xanthones whereas miracil D is a thioxanthone : miracil C is the dihydro derivative of miracil A. Miracil B differs from miracil A in that it contains a chloro substituent in the second benzene ring.

Kikuth and Gönnert (1948) tested these compounds in experimental schistosomiasis in mice and monkeys. When given by mouth these compounds are well tolerated, but when given subcutaneously they cause considerable local irritation which occasionally leads to necrosis at the site of the injection. When given intravenously they are inactive, except miracil D, which is not given therapeutically by this route in man for pharmacological reasons. When given by mouth for the treatment of schistosomiasis in mice miracil B is almost four times as active as miracil C and D and more than eight times as active as miracil A. In the treatment of schistosomiasis in the monkey the relative activities are different. Miracil D is now the best preparation, miracil B produces a chemotherapeutic effect only in doses which cause vomiting, and miracil A, which is only slightly active in mice, shows clear-cut activity in monkeys. Miracil C occupies a midway position in activity in tests both in mice and monkeys.

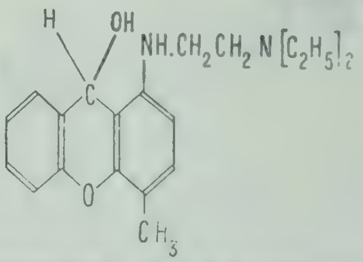
The toxicity of these compounds in mice was studied by Kikuth and Gönnert, who gave a single oral dose, while Sewell (1949) gave oral doses of 5, 10 and 20 mgm. per 20 gm. mouse for four



Miracil A: the hydrochloride of 4-β-diethylaminoethylamino-1-methylxanthone.



Miracil B: the hydrochloride of 8-chloro-4-β-diethylaminoethylamino-1-methylxanthone.



Miracil C: the hydrochloride of 4-β-diethylaminoethylamino-1-methylxanthydrol.

consecutive days. The results, taking into consideration the small number of mice used, show a considerable measure of agreement.

THE TOXICITY OF MIRACIL COMPOUNDS, WHEN GIVEN ORALLY TO MICE

Compound.	Maximum tolerated dose in mgm. per 20 gm.	
	Single dose (Kikuth and Gönnert, 1948).	Four daily doses (Sewell, 1949).
Miracil A . . .	6·7	5
,, B . . .	20	10
,, C . . .	10	5
,, D . . .	13	5–10

The therapeutic activities of miracils A, B and C were studied by Blair *et al.* (1949a) in a small number of Africans infected with *S. hæmatobium.* Daily doses of A and C were given, 100 mgm. by mouth for six days in the first week and 200 mgm. by mouth for six days in the second week, whereas with miracil B only five doses were given in the second week : the total doses for miracils A, B and C were thus 1·8, 1·5 and 1·8 gm. No therapeutic effects were seen but no toxic effects were noted. Miracils A, B, and C can be estimated by the method of Latner, Coxon and King (1947) in the same way as miracil D. After a single dose of 200 mgm. the blood concentration was as follows :—

BLOOD CONCENTRATION OF MIRACIL COMPOUNDS AFTER A SINGLE ORAL DOSE OF 200 MGM. (Blair *et al.*, 1949a)

Compound.	Mgm. per litre at various times after dose.		
	2½ hours.	6 hours.	24 hours.
Miracil A	0·16	0·12	Nil
,, B	0·10	0·28	Nil
,, C	Nil	Nil	Nil

The blood concentrations of miracil A and miracil B are less than those observed after a single dose of miracil D. The blood concentrations of miracils A and B were followed in patients given daily doses of 100 mgm. and 200 mgm. for five days and the urinary concentrations of the drugs were determined on the two days following the end of treatment. The results are shown in the table. The blood concentrations of miracil A and B are lower than those observed in similar patients given miracil D in similar dosage (Hawking and Ross, 1948).

BLOOD AND URINE CONCENTRATIONS OF MIRACILS A AND B DURING FIVE DAILY DOSES BY MOUTH. (Blair *et al.*, 1949)

Daily dose of Compound.	Blood concentrations (mgm. per litre).				Urine concentrations (mgm. per litre).	
	1st Day.		2nd Day.	5th Day.	6th Day.	7th Day.
	2½ hours.	6 hours.	6 hours.	6 hours.		
Miracil A, 100 mgm.	0·20	0·10	0·40	0·32	2·0	Nil
Miracil A, 200 mgm.	0·30	0·46	0·56	0·48	2·7	Nil
Miracil B, 100 mgm.	0·16	0·04	0·08	0·28	1·2	Nil
Miracil B, 200 mgm.	0·24	0·10	0·30	0·34	2·1	Trace

It is possible that by analogy with miracil D larger doses might be tolerated and might be therapeutically effective.

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FILARIASIS

The chemotherapeutic treatment of filarial infections has, up to the present, been unsatisfactory. An enormous number of compounds has been tested, including iodine and salts of gold, copper, tin, zinc, lead, mercury, arsenic, and antimony, thymol, carbon tetrachloride, suramin, pamaquin, aniline dyes, acridine derivatives, emetine, cobra venom and picric acid (Chopra and Rao, 1939, Hawking, 1940). Fresh impetus, however, has been given to the problems of treatment by the large number of American troops infected with filariasis in the Pacific theatre of war and by the extended use as a laboratory animal of the cotton rat, *Sigmodon* sp., which is naturally infected with a filarial worm, *Litomosoides carinii*. An additional experimental animal has thus become available for chemotherapeutic investigations in addition to the dog, infected with *Dirofilaria immitis*.

The evaluation of drugs on standardised laboratory infections, whether in cotton rats or dogs, is by no means easy. Counting the number of microfilariæ in the peripheral blood stream does not give a true appraisal of the drug in every instance, for compounds are known which produce no direct effect upon the microfilariæ but do kill adult worms.

The time which elapses between cessation of treatment and autopsy is also a factor which may influence the evaluation of new compounds.

It is doubtful whether the use of *Dirofilaria immitis* in the dog will ever prove satisfactory for large-scale screening programmes, but there is every possibility that a standardised method may be evolved for testing drugs against *Litomosoides carinii* in the cotton rat. It must be noted that cotton rats inoculated when young sometimes exhibit spontaneous cure. Conclusions from treated infections must therefore be interpreted with due regard to findings in untreated controls (Kershaw and Bertram, 1948).

Heart worm infection in the dog was studied by Itagaki and Makino (1927), who found that, though intravenous injections of sodium antimonyl tartrate caused a disappearance of microfilariæ from the peripheral circulation, the worms which live in the right auricle and ventricle and in the large blood vessels connecting therewith are unaffected. Cheu and Khaw (1935), and Khaw and Cheu (1936), using antimosan and stibophen (Uhlenhuth, Kuhn and Schmidt, 1924), succeeded in killing some adult worms while females which remained alive had their reproductive organs affected, at least temporarily. Thromboses and infarcts were not infrequently caused by dead or debilitated worms, and in curative doses there was considerable fatty degeneration in the internal organs. Popescu (1933) believed that cure could be obtained only with toxic doses, ulceration of the scrotum being an invariable sign of therapeutic efficiency. Concentrated stibophen removed microfilariæ from the peripheral blood stream of dogs, but failed to kill the adult worms.

Studies by Schnelle (1945) and Kingma (1949) suggest that treatment should be continued in dogs for three to four weeks. Stibophen is given daily for seven days: the dose is then increased by 0·5 ml. with each succeeding week of treatment. The relation between the body weight of dogs and the initial dose of stibophen is as follows:—

Weight of Dog.	Initial dose of stibophen in ml.
20 lb. (9 kgm.)	0·5
20–30 lb. (9–13·5 kgm.)	1·0
30–40 lb. (13·5–18 kgm.)	1·5
40–50 lb. (18–22·5 kgm.)	2·0
50 lb. or more (22·5 kgm.)	2·5

Nevertheless Wright and Underwood (1934 and 1936) are of opinion that in countries where dogs are almost invariably incapacitated by filarial infection, stibophen is of considerable value: the majority of infected dogs were restored to usefulness by one or more courses of stibophen, and in only a small number was it impossible to banish microfilariæ from the blood stream. The number of adult worms killed is, of course, uncertain, but histological observations show that antimony acts on the ovaries and uterus of *Dirofilaria immitis* in the same way as it does on schistosomes (Ashburn *et al.* 1945): mercury salts did not give rise to changes either in the ovary or in the ova. More recent observers have confirmed the effect on *Dirofilaria immitis* of stibophen (Brown and Sheldon, 1941), while Brooks (1942) has obtained cures with anthiomaline. Brown and Austin (1939) tested a new antimonial in dogs, stibsol, which is described as sodium antimony-3-catechol-thiosalicylate. It contains 30 per cent. of antimony and 8·5 mgm. of metallic antimony per ml. Eight injections were given in twelve days: for dogs under 22 lb. body weight the first injection was 0·5 ml., rising to 1·5 ml., but dogs over 55 lb. in body weight began with 2·5 ml. and ended with 5 ml. No toxic effects were seen, and microfilariæ were banished from the blood stream. Lawton *et al.* (1945a) tested out an extensive series of antimony compounds in dogs infected with *Dirofilaria immitis* at doses of 0·8 mgm. of metallic antimony per kgm. of body weight. The compounds examined included:—

(1) *Phenolic derivatives*
    Stibophen
    Stibsol (Brown and Austin, 1939)
    Sodium antimonyl 4-tertiary butyl catechol.

(2) *Hydroxy derivatives*
    Tartar emetic.
    Urea antimonyl tartrate.
    Anthiomaline.
    p-Phenetidine antimonyl tartrate.

(3) *Polyhydric alcohol derivatives*
    Sodium antimonyl glycerol.
    ,, ,, DL-erythritol.

(3) *Polyhydric alcohol derivatives—continued.*

Sodium antimonyl adonitol.
,, ,, D-arabitol.
,, ,, xylitol.
,, ,, 2, 4-methylene D-sorbitol.
,, ,, 2, 5-methylene D-mannitol.
,, ,, L-fucitol.

The polyhydric alcohol derivatives were formed by the method of Traube and Kuhbier (1936) whereby an alkaline solution of a polyhydric alcohol reacts with antimony oxide to form water-soluble complex metallic compounds. The ratio between the efficiency of these various antimony preparations against *Dirofilaria immitis* in dogs and the toxicity in mice showed that there was no correlation between the LD 50 dose in mice and the power to destroy microfilariæ in the peripheral blood stream. All the compounds given intravenously to dogs were able to remove microfilariæ from the blood for observational periods of from two to six months, six injections of 0·8 mgm. of metallic antimony per kgm. of body weight being given per week. The average number of injections required to sterilise the blood varied from four in the case of sodium antimonyl 4-tertiary butyl catechol to thirty-two in the case of sodium antimonyl L-fucitol. Some adult worms were killed, while in others, still alive, degenerative changes were visible in the uterine contents. For stibsol (Brown and Austin, 1939) the LD 50 for mice by intravenous injection is 1·11 mgm. per 20 gm. of body weight. When radioactive antimony is injected into dogs in the form of tartar emetic the dead bodies of *Dirofilaria immitis* show a high concentration of antimony (Brady *et al.*, 1945).

In the case of the dog heart worm, microfilariæ are more readily destroyed than adult worms, but with the filaria of the cotton rat the reverse is apparently the case. *Litomosoides carinii* lives in the pleural cavities of cotton rats, thus differing from the canine heart worm, and from human filarial infections. Rose *et al.* (1944) developed an *in vitro* method of testing drugs on worms kept in Simm's physiological serum-salt solution. The method, however, was not successful, for some compounds active *in vivo* showed themselves inactive under the experimental conditions, a result which suggests that quinquevalent antimonials have to be converted into more active compounds in the body. Lawton *et al.* (1945b) treated infected cotton rats with nine different antimonials. After from twelve to eighteen intraperitoneal injections, at a dose rate of 0·8 mgm. of antimony per kgm. of body weight, sodium antimonyl xylitol and the corresponding compounds with arabitol, adonitol and erythritol, were valueless. After forty intramuscular injections at a dose rate of 3·3 mgm. of antimony per kgm. of body weight, *p*-phenetidine antimonyl tartrate, *p*-aminophenyl tartrate and sodium antimonyl catechol thiosalicylate were active and non-toxic. The effect of a number of compounds on *Litomosoides carinii* was tested *in vivo* by Culbertson and Rose (1944), Culbertson and Pearce (1946), and Culbertson *et al.* (1946). Acranil, pamaquin and chloro-N-β-dimethylphenethylamine hydrochloride had some action on the number of microfilariæ in the blood but none on the adult worms. Penicillin and streptothricin had no action either on microfilariæ or adult worms. The only four compounds active in killing the adult worms were (1) neostam (a nitrogen glucoside of sodium *p*-aminophenylstibonate), (2) neostibosan, (3) stibanose or solustibosan, and (4) urea stibamine. Neostam destroyed adult worms in a single dose of 40 mgm. or when given in three doses of 40 mgm. over a week ; neostibosan was given intramuscularly in doses of 40 mgm. on alternate days for fourteen days. At the post-mortem examination the worms were then all dead. Stibanose was given either in doses of 133 mgm. daily for six days a week for two weeks or in doses of 60 mgm. daily for six days to cotton rats. At the end of that time the worms were for the most part dead while the microfilariæ slowly disappeared from the peripheral blood stream : there was no evidence of toxicity. Urea stibamine was given intramuscularly on alternate days in doses of 20 mgm. for eleven days. At the necropsy on the thirteenth day the worms were mostly matted together. Bieter *et al.* (1947) obtained good results in cotton rat filariasis, not only with neostibosan and anthiomaline, but with tryparsamide and melarsen when given in eighteen doses of 100 mgm. per kgm. of body weight. An antimony derivative of sulpharsphenamine also gave promising results in doses of 5 mgm.

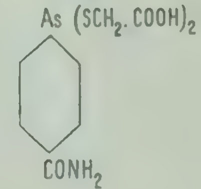
per kgm. of body weight. It has been noted that adult worms are more susceptible to antimony preparations than are immature worms or microfilariæ. It is possible that in man also half-grown worms are less affected by antimony than adult worms : if this is so, chemotherapeutic treatment must necessarily be prolonged.

Brown and Hussey (1947) report that methyl violet is lethal to *Litomosoides carinii* in doses tolerated by the cotton rat.

A number of substances other than those containing antimony have been tested against *Dirofilaria immitis* in dogs. Gentian violet, trypan blue, pamaquin, oil of chenopodium, hexylresorcinol, mercurochrome and emetine hydrochloride have no action in destroying either microfilariæ in the blood or adult worms in the heart (Johnstone, 1936). Underwood (1945) obtained negative results with tryparsamide, acetarsol, sodium cacodylate, antimony thioglycollamide, antimony sodium thioglycollate, bismosol (potassium sodium bismuth-tartrate 10 gm., piperazine 0·3 gm., in aqueous glucose solution to make 100 ml.), bismocymol (a basic bismuth salt of camphocarboxylic acid), bismuth sodium tartrate and ichthargan. Quinquevalent antimonials such as neostibosan have been found to be without action.

Following the suggestion of Kingsbury (1939) that mercury cyanide and mercury succinimide might be of value in human filariasis, Lawton *et al.* (1945) used mercury cyanide, mercury oxycyanide and mercury succinimide in the treatment of four infected dogs. No therapeutic effects were noted even when toxic doses were employed.

Phenyl arsenoxides were, however, found by Maren (1946) to be active against both *Dirofilaria immitis* and *Litomosoides carinii.* The most favourable chemotherapeutic index was obtained with *p*-[bis-(carboxymethylmercapto)-arsino]-benzamide (arsenamide).



This compound is sparingly soluble in cold water, but a solution suitable for intravenous injection can be prepared by dissolving the acid in 0·2 N sodium hydroxide to yield a solution of *p*H 7·0 : a 2 per cent. solution can then be sterilised by filtration and sealed in amber ampoules : the sealed solution keeps for six months at room temperature. The powder is also stable for long periods. It contains 20 per cent. of arsenic. In dogs infected with *Dirofilaria immitis* a 2 per cent. solution was injected intravenously at a rate of 4·5 mgm. per kgm. of body weight or approximately 0·9 mgm. of arsenic per kgm. of body weight (Otto and Maren, 1945). For dogs a dose of 2·25 mgm. (0·45 mgm. As) per kgm. of body weight is recommended daily for fourteen days (Otto and Maren, 1948). The toxicity is the same as that of mapharside (Otto and Maren, 1947) but red cells do not interfere with the action of arsenamide (Otto and Maren, 1949).

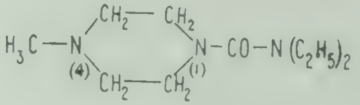
The distribution of radioactive tervalent arsenic in cotton rats infected with *Litomosoides carinii*, following the intraperitoneal injection of sodium arsenite, shows that these filaria have a specific affinity for arsenic. The largest amount was found in the kidney, followed by the liver, filarial worms, skin, spleen and lung ; no special concentration was found in the thyroid (Lawton *et al.*, 1945b). Neither penicillin nor the other antibiotics have any action *in vivo* on filariæ. Graessle and Bugie (1946), however, made the curious discovery that crude penicillin, though almost inert against bacteria is nevertheless capable of killing *Dirofilaria immitis in vitro*. This action is due to some impurity.

Piperazine Derivatives

The effect of various piperazine derivatives against filarial infection in the cotton rat and dog have been studied by Hewitt and his colleagues (1947a, b and c). Particulars of piperazine derivatives having some action in the cotton rat are shown in the table. The piperazine nucleus itself is without activity and so are its components. The addition of the carbethoxy radical in position 1, with various substitutions in position 4, produced a number of compounds with high microfilarial activity in the cotton rat. Alkyl radicals in position 4 produced a number of compounds with high microfilaricidal activity. Alkyl radicals in position 4 retained high activity as far as the propyl and *iso*propyl derivatives but butyl substitutions were much less active. Toxicity increases as the alkyl chain is lengthened. Retaining the carbethoxy group in position 1, and two other substitutions in position 4, guanyl and carbethoxy, maintained activity.

The only compounds lacking the carbethoxy group which nevertheless showed activity against microfilariæ were 1-ethylcarbamyl-4-methylpiperazine hydrochloride, 1-di*iso*propylcarbamyl-4-methylpiperazine hydrochloride, 1-dimethylcarbamyl-4-methylpiperazine hydrochloride and 1-diethylcarbamyl-4-methylpiperazine hydrochloride. The two latter compounds are the most active so far found. A measurable reduction in microfilariæ usually occurred within twenty-four hours after the first dose, and this reduction was maintained so long as treatment continued. Recurrences of microfilariæ frequently took place after cessation of treatment.

In dogs the only two compounds which produced measurable reductions in microfilariæ in tolerated doses were 1-carbethoxy-4-methylpiperazine hydrochloride and 1-diethylcarbamyl-4-methylpiperazine hydrochloride (hetrazan, banocide, RP 3799). The oral or intraperitoneal doses of the first compound (25 to 100 mgm. per kgm. twice daily) which produced reductions in microfilariæ invariably caused salivation, nausea, and muscular weakness. The latter compound is far better tolerated and after doses ranging from 3 to 100 mgm. per kgm. the microfilarial count in the blood of dogs falls precipitately in twenty-four hours. It becomes negative or remains at a very low level for as long as treatment is continued. There is, however, failure to kill all adult worms in all animals. The best results in cotton rats were given by treating the animals three times daily for thirty days with 10 to 25 mgm. per kgm.



1-Diethycarbamyl-4-methylpiperazine ("hetrazan")

Comparative Microfilaricidal Activity and Toxicity of Eighteen Piperazine Derivatives in Cotton Rats, using 1 - Diethylcarbamyl - 4 - Methylpiperazine Hydrochloride (Hetrazan) as a Standard

Compound.	Minimum effective dose against microfilariæ (mgm./kgm.).	L.D. 50 Mice I.P. (mgm./kgm.).	Approximate 84-L. equivalent.	Approximate chemotherapeutic index.
1-Phenylpiperazine hydrochloride	50 I.P.	140	0·06	2·8
1-Carbethoxypiperazine hydrochloride	25 I.P.	275	0·125	11·0
1-Methyl-4-(4′-morpholine carbamyl)-piperazine hydrochloride	12·5 I.P.	?	0·25	?
1-Carbethoxy-4-methylpiperazine hydrochloride	6·25 I.P.	550	0·5	88·0
1-Carbethoxy-4-ethylpiperazine	50 I.P.	175	0·06	3·5
1-Carbethoxy-methylpiperazine	100 I.P.	?	0·03	?
1-Carbethoxy-4-propylpiperazine hydrochloride	25 I.P.	87·5	0·125	3·5
1-Carbethoxy-4-*iso*propylpiperazine hydrochloride	25 I.P.	100	0·125	4·0
1-Carbethoxy-4-butylpiperazine	50 I.P.	125	0·06	2·5
1-Carbethoxy-4-s. butylpiperazine	100 orally	175	0·05	1·75 *
1-Guanyl-4-carbethoxy-piperazine sulphate	50 I.P.	285	0·06	5·6
bis-(4-Carbethoxy-1-piperazine)-methane	25 I.P.	175	0·125	7·0
1, 4-Dicarbethoxypiperazine	100 I.P.	500	0·03	5·0
1, 4-Dicarbethoxy-2-methylpiperazine	200 orally	375	0·025	1·9 *
1-Ethylcarbamyl-4-methylpiperazine hydrochloride	6·25 I.P.	2,250	0·5	360·0
1-Dimethylcarbamyl-4-methylpiperazine hydrochloride	6·25 I.P.	310	0·5–1·0	48·0
1-Di*iso*propylcarbamyl-4-methyl-piperazine hydrochloride	6·25 I.P.	160	0·5	25·6
1-Diethylcarbamyl-4-methylpiperazine hydrochloride	3·13 I.P.	285	1·0	91·0

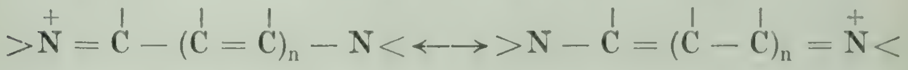
* Probably not a true value, since the LD 50 was determined by intraperitoneal injections and the minimum effective dose by oral administration.

I.P. = Intraperitoneal injection.

Cyanine Dyes

Welch *et al.* (1947a and b) have reported on the activity of certain cyanine dyes in the chemotherapeutic treatment of *Litomosoides carinii* in the cotton rat.

1-Amyl - 2, 5 - dimethyl - 3 - pyrrole (1, 6 - dimethyl - 2 - quinoline) dimethincyanine chloride (No. 348) was found to kill all worms in the maximum tolerated doses used. Intraperitoneal injection of 0·1 mgm. per kgm. at eight hours interval for eighteen doses regularly kills all filarial worms. *In vitro* a number of cyanine dyes are found to inhibit the oxidative mechanism of the worms. No. 348, for instance, causes inhibition in concentrations ranging from 1 : 6,000,000 to 1 : 25,000,000 while aerobic glycolysis increases and glycogen synthesis decreases. Under anaerobic conditions no effect on glycolysis was observed. Only with concentrations from 1,000 to 2,000 times greater is the oxygen consumption of mammalian tissue slices affected, Bueding (1947). All cyanine compounds active *in vitro* were found to be active *in vivo*. Almost all cyanines and many styryl dyes inhibit filarial respiration *in vitro*, the characteristic chemical grouping being the amidinium-ion system in which a positively charged quaternary nitrogen is linked to a tertiary nitrogen by a conjugated



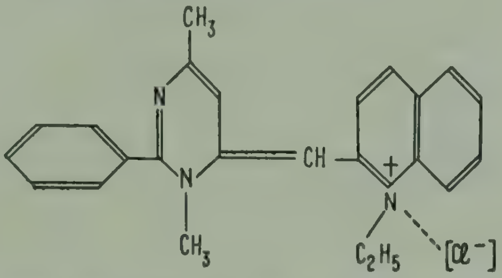
$$>\overset{+}{N}=\overset{|}{C}-(\overset{|}{C}=\overset{|}{C})_n-N<\longleftrightarrow>N-\overset{|}{C}=(\overset{|}{C}-\overset{|}{C})_n=\overset{+}{N}<$$

chain consisting of an uneven number of members. One or both of the nitrogens may be part of a heterocyclic ring but activity is not restricted to any particular ring. Any modification which destroys the possibility of amidinium-ion resonance in the compounds causes a disappearance of high activity both *in vitro* and *in vivo*. Antifilarial activity is increased by substitution of alkyl or alkoxy radicals in position 6 of the quinoline ring, but the compounds then develop increased irritant properties (Cranston *et al.*, 1947).

Cures are best obtained *in vivo* by intravenous or intraperitoneal injection. Subcutaneous or intramuscular inoculation causes local tissue damage. With No. 348 a total of 1·8 mgm./kgm. intraperitoneally was required when administration was at eight hours intervals for six days while a total of 1 mgm. per kgm. was curative when the drug was given once daily for five days ; cures could also be obtained when animals were given 1·35 mgm./kgm. in a single dose.

Good results were also obtained by the intravenous injection of infested cotton rats with a compound No. 863 which is probably

1′-ethyl-3, 6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine chloride, although an isomeric structure is possible.



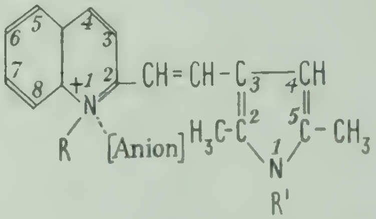
Cures were almost invariably produced in cotton rats when No. 863 was given intravenously in doses of 1 mgm. per kgm., repeated for from three to six times at intervals of one, three or even seven days. No toxic effects were seen unless individual doses of 10 mgm. or more per kgm. were administered. The only effect in dogs and monkeys on repeated injections was mild and reversible renal damage. During intravenous injection there is a transient fall in blood pressure. Spectrophotometric determination of No. 863 at 494 mμ shows that after intravenous administration in dogs it rapidly disappears from the blood stream while urinary excretion rarely exceeds 5 per cent. The drug is apparently metabolised in some way but can be shown to be concentrated in the kidneys, especially in the convoluted tubules. In man intravenous injection causes only slight fall in blood pressure and some tachycardia (Valk *et al.*, 1947).

Further cyanine derivatives have been studied by Wright *et al.* (1947). Diquinolines of the type of 1, 1′-dimethyl-2, 2′-cyanine chloride had only a slight action on *Litomosoides carinii* in the cotton rat: 1, 1′-dimethyl-2, 2′-carbocyanine chloride was more active but the most effective compounds were 1, 1′-di-β-ethoxyethyl-2, 2′-carbocyanine chloride and the corresponding *p*-toluene sulphonate derivative. When given to cotton rats every eight hours for eighteen days in single doses of 0·0167 gm. the first compound was completely curative, the chemotherapeutic index being at least eighty while the *p*-toluene sulphonate derivative had a chemotherapeutic index of 100. Intraperitoneal injections daily for six days were also curative. The LD 50 of the drug given intravenously to rabbits, rats and guinea-pigs is from 4·5 to

5·5 mgm. Dogs have been given 1 mgm. per kgm. of body weight two or three times weekly on from fifty to eighty occasions without any ill effects, but if six doses are given weekly death occurs after about twenty doses. In man the only toxic change noted after very high dosage is some reversible kidney damage (Peters *et al.*, 1947a). In dogs and monkeys the only lesions are slight cloudy swelling in the cells of the convoluted tubules (Peters *et al.*, 1947b). Cyanines seem of little value against *D. immitis* infections in dogs.

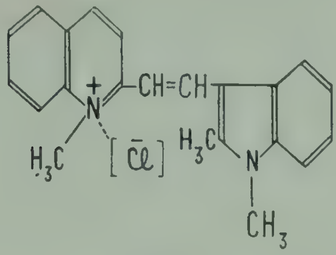
Peters *et al.* (1948) attempted to correlate the *in vitro* effect on respiration with the *in vivo* action of cyanines on *L. carinii*. All compounds active *in vivo* inhibit respiration *in vitro*, but the reverse is not true. The relative antifilarial activity of (3-pyrrole) (2-quinoline) dimethinecyanines against *L. carinii* in cotton rats is shown in the table :—

THE RELATIVE ANTIFILARIAL ACTIVITY OF (3-PYRROLE) (2-QUINOLINE) DIMETHINCYANINES AGAINST *L. carinii* IN COTTON RATS (Peters *et al.* 1948)



No.	Substituent Group.						Minimum curative dose, mgm. per kgm.	Maximum curative dose, mgm. per kgm.	Therapeutic index.	*In vitro* index.
	1		R′	6	7	8				
	R	Anion.								
348	CH_3	Cl	C_5H_{11}	CH_3	—	—	0·20	2·0	10	1·0
713	CH_3	Cl	C_6H_5	OCH_3	—	—	0·80	8·0	10	0·25
963	C_2H_5	Cl	C_5H_{11}	CH_3	—	—	0·30	4·0	13	1·5
964	C_3H_7	Cl	C_5H_{11}	CH_3	—	—	0·15	2·0	13	0·8
943	CH_3	Cl	C_5H_{11}	—	—	—	0·40	4·0	10	1·0
967	CH_3	Cl	C_5H_{11}	OCH_3	—	—	0·40	6·0	15	1·0
712	CH_3	Cl	$C_{12}H_{25}$	CH_3	—	—	0·40	$<$4·0	$<$10·0	0·1
714	CH_3	Cl	$C_6H_4OC_2H_5$	CH_3	—	—	0·30	$<$3·0	$<$10·0	0·6
798*							0·80	8·0	10	0·5

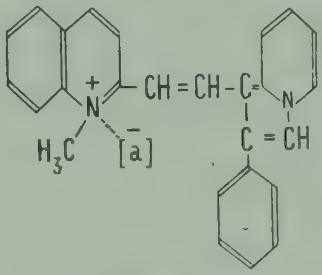
* See formula on next page.



798

1-Amyl-2, 5-dimethyl-3-pyrrole (1, 6-dimethyl-2-quinoline) dimethincyanine chloride, No. 348, was completely curative in the maximally tolerated dose against *L. carnii.*

Among (3-pyrrole) (4-quinoline) dimethincyanines greater activity was observed when an amyl radical was attached to the pyrrole-N than when a phenyl group was present. Diquinoline cyanines and also a number of cyanines in which the quaternary nitrogen was present in a quinoline ring and the tertiary nitrogen in a heterocyclic ring other than quinoline or pyrrole were active. Compound 835, like 863, had a chemotherapeutic index of 10·0.



835

Pyrrole-pyridine cyanines are of interest because of the deleterious effects of replacement of an amyl group on the pyrrole-N by a phenyl radical. Pyrrole-benzimidazole and pyrrole-benzothiazole cyanines are not outstandingly active either *in vivo* or *in vitro.*

Many dyes resemble the cyanines in possessing a tertiary and quaternary nitrogen separated by a " conjugated " chain of atoms. Although some of these dyes possessed an *in vitro* action, none are active *in vivo.*

The Mode of Action of Cyanine Dyes

The mode of action of cyanine dyes was further studied by Bueding (1949), who has investigated the metabolism of *Litomo-*

soides carinii. Oxidative metabolism is essential for the survival of the filarial worm, but the various cyanine dyes inhibit this respiration in very low concentrations.

High antifilarial activity *in vitro* is not restricted to any particular ring, but any structural modification which destroys the possibility of amidinium ion resonance causes a disappearance of high antifilarial activity both *in vivo* and *in vitro*. The cyanine dye 1-amyl-2, 5-dimethyl-3-pyrrole (1, 6-dimethyl-2-quinoline) dimethincyanine chloride in a dilution of 1 in 40,000,000 significantly inhibited the oxygen uptake of filariæ.

Although the inhibition of respiration results in a compensatory increase in glycolysis, anaerobic reactions appear to be inadequate to maintain the organism alive, because the administration of only three to four higher doses of cyanines produces the death of the filariæ (Welch *et al.*, 1947 ; Wright *et al.*, 1947). This behaviour of *L. carinii* contrasts with that of *Schistosoma mansoni :* although the cyanines inhibit the oxygen uptake of this helminth, the parasite survives. There are indications that other parasites can survive under anaerobic conditions (von Brand, 1946).

Inhibition of filarial respiration by cyanines increases the proportion of the utilised glucose converted to lactic acid. *L. carinii* is the only helminth which has lactic acid as the main end-product of anaerobic carbohydrate metabolism : in addition there is a decreased rate of acetic acid production and of polysaccharide synthesis. The intraperitoneal injection of cyanine dyes into infected cotton rats in sublethal doses also increases aerobic glycolysis and decreases the oxygen uptake, although the worms remain alive and motile : in addition the cyanines have no significant effect on anaerobic glycolysis : lactic acid production in an atmosphere of nitrogen is practically the same whether cyanine dyes are present or not. The inhibitory effect of cyanine dyes on the activity of cytochrome oxidase is observed only in concentrations of $2{\cdot}6 \times 10^{-4}$ M, a concentration 4,000 times higher than that required to inhibit the respiration of the filariæ.

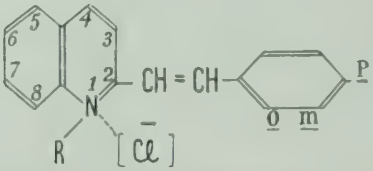
The observed inhibitory effect of the cyanines on filarial respiration is probably explained by an interference with a respiratory enzyme or coenzyme concerned with the transfer of electrons, rather than by an inhibition of a specific dehydrogenase. This is indicated by the fact that the cyanines inhibit the oxygen uptake of filariæ in the absence of glucose, even when the polysaccharide stores of the worms have been depleted by previous anaerobic incubation. This group of compounds therefore decreases the respiration of the worm, regardless of whether carbohydrate or other substrates are oxidised. In contrast to filariæ, concentrations of cyanine dyes up to 1×10^{-5} M did not affect the oxygen uptake of slices or homogenates of mammalian tissues. As the activity of cytochrome C and cytochrome oxidase also are not affected, it is evidence that cyanines inhibit in these filarial worms an enzyme system which plays no *rôle*, or only a minor one, in mammalian tissues.

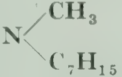
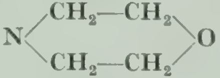
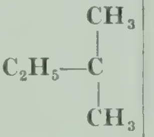
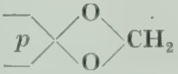
Styryl Quinoline Compounds

Styryl quinoline compounds possess a quaternary and a tertiary nitrogen connected by a carbon chain whose bonds are alternately single and double. They differ from the cyanines in that the tertiary nitrogen is not a member of the heterocyclic ring but rather is a *para*-amino nitrogen.

Their chemotherapeutic activity in infected cotton rats has been studied by Bieter *et al.* (1947), Wright *et al.* (1947, 1948) and Cranston (1947). Compound 350, where $R = C_2H_5$, $p = N(C_2H_5)_2$ and $6 = CH_3$, has a maximum tolerated dose of $<8{\cdot}0$ mgm. per kgm. of body weight and a minimum curative dose of 1·6 mgm. per kgm.: its chemotherapeutic index is thus $<5{\cdot}0$. All other compounds had a methyl rather than an ethyl substituent on the quinoline-N. Variants involved particularly the amino-nitrogen where di-*iso*-propyl substitution yielded higher activity than di-*n*-propyl or di-*n*-butyl substitution, but di-*n*-amyl substitution yielded a compound of considerably less potency. One compound (K-188) contained no tertiary nitrogen and hence no resonating system between two nitrogens. This compound had an *in vitro* index of 0·2 and was the only compound which had an index of significance despite the absence of the resonating amidinium ion system.

THE RELATIVE ANTIFILARIAL ACTIVITY *in vitro* AGAINST *L. carinii* IN THE COTTON RAT OF STYRYL-QUINOLINE DYES
(Peters *et al.*, 1949.)



No.	Substituent groups.			*In vitro* chemotherapeutic index.
	R	p	6	
350	C_2H_5	$N(C_2H_5)_2$	CH_3	2·0 (*in vivo* index = < 5·0)
759	CH_3	$N(C_3H_7)_2n$	CH_3	0·4
763	CH_3	$N(C_3H_7)_2(iso)$	CH_3	1·0
760	CH_3	$N(C_4H_9)_2$	CH_3	0·3
762	CH_3	$N(C_5H_{11})_2$	CH_3	0·1
764	CH_3	 $N(CH_3)(C_7H_{15})$	CH_3	0·5
757	CH_3		CH_3	0·5
773	CH_3	$N(C_2H_5)_2$	 $C_2H_5—C(CH_3)_2$	1·0
K-188	CH_3		—	0·02

Bancroftian Filariasis in Man

The filarial infections of man include those due to *Wuchereria bancrofti*, *W. malayi*, *Mansonella ozzardi*, *Loa loa* and *Onchocerca volvulus*.

Early attempts to treat filarial infections due to *Wuchereria bancrofti* in man were entirely unsuccessful (Anderson, 1924) though Dalal (1927) thought that neoarsphenamine reduced the fever, while Chopra and Rao (1929) believed that tryparsamide cleared up the chyluria. Rogers (1919 and 1920) used sodium antimonyl tartrate on patients with *Wuchereria bancrofti* infections;

doses up to 5 ml. of a 2 per cent. solution were given, a total of 1·8 gm. being administered up to five and a half months. A considerable fall occurred in the microfilarial counts, and in view of the fact that the embryos decline over a period of some months after the cessation of antimony treatment these patients may well have been cured. Roger's work was soon confirmed. In 1920 Das reported on eight cases treated with from twelve to thirty-seven doses of sodium antimonyl tartrate with complete disappearance of large numbers of microfilariæ in five, and considerable decrease in the numbers of the other three. Day (1921), also in Egypt, found complete disappearance of microfilariæ from the blood with large doses of the same drug. In 1920, in cases of filarial fever, Rogers (1920), Das (1920) and Bär (1924) noted cessation of the attacks under antimony treatment. Roy and Bose (1922) reported on the use of sodium antimony gluconate in fifty cases of elephantiasis. The circumference of the limbs decreased in all and in some to a material degree. Low and O'Driscoll (1921), however, failed to note any disappearance of microfilariæ in two patients given 17·5 gr. (1·13 gm.) of tartar emetic. Chopra and Rao (1929) found that stibophen caused a temporary disappearance of microfilariæ for some days and also cured chyluria. De Choisy (1937) gave one patient 35 ml. of anthiomaline (lithium antimony thiomalate): some improvement was seen but no filarial counts were made on the patient's blood before, during, or after treatment. Poynton (1938) injected anthiomaline directly into the enlarged lymph nodes: the febrile attacks, it is claimed, were reduced in numbers and in intensity. Hawking (1940) found no action on the microfilariæ in seven cases treated with stibophen and ten cases treated with anthiomaline for periods of seven to forty-nine days: the total dosages of stibophen varied from 23·5 to 119·0 ml., that of anthiomaline from 7 to 25 ml.

More recently, intensive antimony treatment has been used experimentally. Brown (1944) used a solution of anthiomaline, 1 ml. containing 60 mgm. of the compound or 10 mgm. of antimony: twelve adult patients received doses of 3 ml. after a preliminary dose of 1·5 ml. The dosage and percentage reduction of microfilariæ in the peripheral blood stream was as follows:—

Age.	Wt. in lbs.	Number of days of treatment.	Total dosage ml.	Percentage reduction in microfilariæ.
23	118	13	19·7	89·3
70	126	17	43·5	97·1
11	68	28	48·5	99·9
19	132	20	55·5	0
28	124	12	27·0	0
17	140	19	49·0	96·9
16	86	18	35·5	85·5
11	76	18	32·0	100·0
21	96	7	15·0	99·3
28	155	26	76·5	100·0
16	100	16	29·5	86·0
31	125	17	42·5	85·5

Vomiting and epigastric pain occurred in five patients : vomiting was most frequent two to three hours after injection though sometimes it was delayed till ten or twelve hours. Generally, vomiting began after several injections, but in some cases it ceased despite continuance of treatment. Rest in bed did not prevent vomiting. Pain in the epigastric region occurred both before and after vomiting. Two patients had fever and sore buttocks : one had arthritis, while six had itching rashes on the lateral aspect of the fingers and thumbs, forehead, chest, and abdomen : small raised papules 1 to 2 mm. in diameter were noticeable. A reduction in the number of red cells from 500,000 to 1,000,000 per c.mm. was noted, but no change in the hæmoglobin concentration. Four and a half to five months later all the patients were in good health but there was no reduction in the size of the enlarged lymph nodes or of the enlarged scrotum possessed by one patient. Brown and Thetford (1946) were able to follow up patients treated with anthiomaline for two years. No evidence of inflammation or incipient elephantiasis was noted. Preparations of anthiomaline from France and the United States of America were equally effective, but the American product was less toxic. In one series of eighteen patients treated for from seven to twenty-eight days four became negative while eleven showed a reduction of at least 90 per cent. in the microfilariæ present in the blood stream. Culbertson *et al.* (1946) treated twenty patients with a six per cent. solution. The first dose was 1·5 ml. and thereafter 3 ml. daily; four patients continued for twenty days, but in the others treatment was stopped in from seven to eighteen days, owing to severe reactions, anorexia, nausea, vomiting, and headache. In a second series of ten treated with anthiomaline from another source, the first dose was 1 or 2 ml., 3 to 5 ml. on the second day, and 4 to 8 ml. on the third day. Only two patients tolerated the drug well, receiving 53 and 70 ml. in fourteen days: in all the others the reactions were severe. In the first series eight months after treatment, two patients, both of whom had previously received neostibosan, were free from microfilariæ and seven of the remaining eight had lost over 50 per cent. of the microfilariæ initially present. In the second series two were negative seven months after treatment and five had lost 50 per cent. of their microfilariæ.

Culbertson *et al.* (1946) treated fifteen patients with stibophen (6·3 per cent. solution). The first dose was 5 ml., the second, third and fourth 10 ml., and in some cases 15 ml. on the fifth day. This dosage was too heavy and no drug was given for three days. Thereafter 4 to 6 ml. were given daily up to fourteen days, but only four patients could stand this full course. Treatment, owing to reactions, was stopped in one case on the sixth day. All patients reported nausea, vomiting, abdominal or bone pain, headache, fever, and salivation, several had anorexia and a persistent rash. By the eighth month after treatment no patient was free from microfilariæ, but four of fifteen patients has lost over 90 per cent. of circulating embryos.

Culbertson, Rose and Oliver-González (1945a and b; 1946) studied the effect of neostibosan in human filarial infections due to *Wuchereria bancrofti*. Thirty-five patients were treated: they were divided into three groups, which received the following dosage:—

Group	Number of Patients.	Dosage.
I	20	4·6 to 10·5 gm. in from thirty-three to fifty-six days.
II	10	As for Group I, but as results were unsatisfactory ten months later, 5·9 to 12·5 gm. in fourteen days.
III	5	9·5 to 15·5 gm. in from thirteen to fourteen days.

All injections were given intravenously: the first injection of 0·1 gm. was gradually increased up to 1 gm. per day. The following results were obtained :—

Group I. Fifteen patients had lost all microfilariæ after fifteen months ;

Three patients had lost a considerable number of microfilariæ ;

Two patients showed little or no change after nine months.

Group II. Three had become negative five months after their second course ;

Six showed a considerable reduction ;

One showed no change.

Group III. Two had become negative after six months.

Three showed a reduction in filarial counts by half.

Urea stibamine was also used intensively on six patients, two different preparations being tested. On the first day 0·1 gm. was given of both preparations, followed in the case of one preparation by 0·6, 0·75 and 0·9 gm., while in the case of the other preparation the maximum dose was 0·525 gm. The maximum number of days treatment was seventeen, the minimum eleven. One preparation caused hardly any reaction, the other produced nausea, vomiting, abdominal pain, headache and salivation. Ten months after treatment four patients were quite negative and one had lost 83 per cent. of the original number of embryos.

Among fifteen controls none were free from microfilariæ in from fourteen to seventeen months, but in eleven patients the numbers of microfilariæ had increased. Half the patients had nausea and vomiting and in two these symptoms were severe but not so severe as to necessitate a termination of treatment. These results suggest that quinquevalent antimonials may have some action in infections due to *Wuchereria bancrofti*, if large doses are given for a short period.

Of four patients treated with tartar emetic one became free from microfilaria by the seventh month, the other three were only slightly improved. From 0·73 to 0·88 gm. of antimony were given in fourteen days: one patient had severe shock and all had headache, abdominal pain, bone pain and rashes from the sixth to eighth day.

Apart from antimony compounds, Culbertson *et al.* (1946) have studied the effects of the tervalent melarsen oxide which has been used in the treatment of trypanosomiasis (p. 384). Eighteen patients received the drug, three orally in doses of 50 mgm. three times daily for one week and, after a few days rest, for several additional days, the remainder intravenously, 7·5 or 10 mgm. being dissolved in propylene glycol for seven or nine successive days. Eleven of the fifteen patients treated intravenously with melarsen oxide showed no ill effects; of the remaining four, one had generalised adenopathy and malaise for two or three days after the final injection on the seventh day, three others had occipital headache on the eighth or ninth day of treatment and two of these developed an arsenical encephalopathy from which they eventually recovered. Of the three patients who received melarsen oxide orally one had no ill effects but the other two had severe headache and abdominal pain with dermatitis. Of the eighteen patients, six lost all microfilariæ by the sixth or seventh month after treatment, while four had lost over 70 per cent.

An interesting sequela observed by Culbertson *et al.* (1946) in adult male patients was the occurrence in many, towards the end of treatment, of scrotal reactions, painful swelling of the testis, spermatic cord or epididymis, and the subsequent development in the scrotum of nodules sometimes 1 cm. or more in diameter. These swellings usually subsided within a week or so, but the nodules persisted for several months. Sections of a nodule showed one or more adult filarial worms surrounded by an inflammatory area or lying in distended vessels enveloped by thrombi. Histological appearances suggested that the worms were recently dead.

Stilbamidine was found to be without effect in a patient infected with *Wuchereria bancrofti* (Snapper and Merliss, 1946). Gentian violet, when given orally, was thought to have a very slight action in infections due to *W. bancrofti* (Ashford and Snyder, 1933), but Snapper and Merliss, who gave gentian violet in a continuous intravenous drip for nine days found no effect on the microfilaria count of *W. bancrofti;* the same patient, however, was infected with *Mansonella ozzardi* (*Microfilaria demarquayi* and *Mf. tucumana*), and these microfilariæ did show a progressive decrease, though they did not disappear from the peripheral blood. Despite

1·725 gm. of gentian violet in nine days, the only toxic symptoms were a rise in temperature and purple vision.

It is obvious that while antimony preparations are of value in large doses for filariasis in man, there is still room for a further advance in the treatment for filarial infections.

Hetrazan, 1-diethylcarbamyl-4-methylpiperazine hydrochloride, has been used by Stevenson *et al.* (1947a and b) in the treatment of twenty-six patients in Puerto Rico infected with *W. bancrofti*. The drug was given by mouth in doses of from 0·5 to 2 mgm. per kgm. of body weight three times a day for from three to twenty-two days : in one case a dose of 2 mgm. per kgm. was given for twenty-two days. In all cases there was a remarkable diminution in the number of microfilariæ in the blood stream. Nine patients became negative on the second day of treatment and in thirteen the blood was negative from ten to eighty-three days after treatment. In all the others there were only a very few microfilariæ left eight to eighty-three days after treatment. It is therefore suggested that hetrazan has a lethal action on the adult worms. There were no toxic reactions except that in those given maximal doses there was usually slight fever during the first forty-eight hours of treatment, and in four cases there was considerable enlargement of the lymph nodes draining the extremities and of the spermatic cord, associated with regional lymphadenitis. Hetrazan appears to be the most active filaricidal drug yet used in man (Hawking and Laurie, 1949).

Cyanine dyes, so far as they have been tested, have no action on *W. bancrofti* (Peters *et al.*, 1948).

PROPHYLAXIS OF FILARIASIS

The possibility of prophylaxis of filariasis in man by means of hetrazan was suggested by Stefanopoulo and Schneider (1948) and carried out in a limited number of cases by the former (Stefanopoulo, 1949). Kershaw *et al.* (1949) have shown that in the cotton rat such a prophylaxis can be obtained by injections of *p*-melaminyl phenyl stibonate. This compound is one of a series of quinquevalent antimonials prepared by Friedheim *et al.* (1947) for the prophylaxis of trypanosomiasis. Preliminary experiments show that after doses of 250 to 1,000 mgm. per kgm.

of body weight prophylactic action continued for twenty-one days. Stilbamidine and suramin are inactive at doses of 50 mgm. and 500 mgm. per kgm. respectively after shorter intervals.

THE CHEMOTHERAPY OF OTHER FILARIAL INFECTIONS

Unpublished observations in West Africa on patients with *Loa loa* infections showed that among the arsenoxides *p*-arsenophenylbutyric acid caused the disappearance of microfilariæ from the peripheral blood stream, but after from ten to fifteen days they returned. Rose and Culbertson (1946) have used neostibosan, intravenous injections being given twice daily for two weeks. A girl of nine years received 9·4 gm., while two adults were given 11·1 gm. and 16·4 gm. : the incidence of swellings was reduced though cure was not attained.

The chemotherapy of infections due to *Onchocerca volvulus* is practically non-existent. Sharp (1927) claimed some success with a gold preparation described as B 1916. Adams (1938) reported that neostibosan caused the disappearance of microfilaria from the skin of a European, while Harris (1940), after excision of the nodules, used sodium antimonyl tartrate with success in twelve of sixteen cases, improvement being judged by the disappearance of microfilariæ from the skin and a decrease in the eosinophilia. Marill and Alcay (1941) and Enzer (1942) found euflavine, tryparsamide, and suramin useless, unless combined with shock therapy. Hewitt and Mazotti (1948), Frémont (1949) and Hawking and Laurie (1949) report the failure of hetrazan in onchocerciasis. In West Africa, injection of neostibosan, after excision of nodules, appeared to reduce temporarily the number of microfilariæ in the skin. Similar results were obtained by Culbertson (1947). Ruiz Reyes (1947) found suramin, 1 gm. intravenously once a week, quite valueless.

In the Anglo-Egyptian Sudan reactions have followed the intravenous injection of suramin for treatment of onchocerciasis, and the same is true of America and the Belgian Congo. A dose of 1 gm. twice weekly has been given; the minimum total dose is at least 5 gm. Antihistamine drugs have been recommended (Pieltain, 1947).

Mazzotti (1948a), however, obtained results with hetrazan in onchocerciasis in America. The dose was 2 mgm. per kgm. three times a day, but on the first day only one injection was given and on the second day two injections. Treatment was continued for twenty-one, thirty or forty-five days. Nodules excised showed dead worms and skin biopsies were positive only in 10 per cent. of cases, and then only one to four microfilariæ were seen, immobile and probably dead. Patients with ocular lesions showed disappearance of microfilariæ from the fluids in the anterior and posterior chambers. In the Anglo-Egyptian Sudan severe reactions have been noted in patients given hetrazan for onchocerciasis. The symptoms include skin rashes, pruritus, swelling of the lymph nodes and œdema : sometimes the scrotum has become swollen and ulceration has occurred : the toxic symptoms suggest a Herxheimer type of reaction.

Mazzotti (1948b) obtained entirely negative results in patients infected with *Mansonella ozzardi*.

Attempts have been made to study the effects of chemotherapeutic drugs on *Icosiella neglecta*, a filarial worm of the frog *Rana esculenta*. Lagrange (1949) finds that intravenous injections of a 2·5 per cent. solution of methyl violet to give a dose of 12 mgm. per kgm. of body weight kills the adult worms, although microfilariæ are still present in the peripheral blood. Similar results were obtained with an arsenical, dipharsine, described as hydroxyamino-phenyldichlorarsine chlorohydrate.

In Indian cattle *Parafilaria multipapillosa* causes slightly raised subcutaneous nodules which break down with hæmorrhage. Intravenous injections of 100 ml. of 1 per cent. potassium antimonyl tartrate were found by Gulati (1934) to have a curative action.

Filarial dermatosis in the sheep, due to *Elæphora schneideri*, was described by Kemper (1938) as a circumscribed dermatosis of the forehead of the sheep, sometimes extending to the nostrils and lips. Although promising results have been obtained with stibophen and anthiomaline, the most satisfactory follow from six to eight intravenous injections of tartar emetic, one injection a week of 0·3 gm. in 30 ml. of water and 4 ml. of 50 per cent. glucose solution (Kemper and Roberts, 1946). Toxic reactions occur after the administration of from 30 to 40 ml.

Hetrazan has been used in the treatment of *Loa loa* infections in twenty patients from Africa. The drug was given orally by

Stefanopoulo and Schneider (1948) in doses of from 3 to 6 mgm. per kgm. of body weight daily for seven to ten days. A few hours after treatment patients had formication accompanied by pruritus, temporary œdema and rarely a generalised erythema. Very rarely there was fever associated with nausea, arthralgia and diarrhœa. These reactions disappeared in forty-eight hours. The general improvement was rapid ; headache, œdema and pruritus ceased and microfilariæ disappeared from the circulation. In only one case were microfilariæ still present after seven days' treatment, and even in this instance they disappeared later. Not infrequently, two to four months after the first course there is a recrudescence of symptoms, pruritus especially, but no recurrence of microfilariæ. To avoid this recurrence, two to four courses of 0·4 gm. are given daily for ten days, each course being separated by an interval of three to four weeks. Thus long periods of freedom from symptoms have been obtained and possibly some cures.

Hawking *et al.* (1948) believe that hetrazan does not have a direct lethal action on either adult worms or microfilariæ, but that the microfilariæ are modified or opsonised so that they are readily phagocytosed. Stefanopoulo and Schneider (1948) noted the expulsion of dead worms, and Murgatroyd and Woodruff (1949) observed dead *Loa loa* in seventeen patients.

Arsenamide has been used against *Loa loa* infection and by Thetford *et al.* (1948) to treat seven patients infected with *W. bancrofti*. The sodium salt was given intravenously as the sodium salt in a 2 per cent. solution in buffered phosphate (*p*H 7·0) in daily doses of 0·05 ml. per kgm. of body weight (= 0·2 mgm. As) for fifteen days.

In four cases microfilariæ were removed from the blood stream and did not return ; in three very small numbers were found at subsequent examinations, in one instance as late as the seventeenth month, but in all except this case the final result was complete absence of microfilariæ. Slight toxicity was noted in three patients and local reactions in four others. Two patients were also apparently cured by mapharsen ; in one case 0·06 gm. was given daily for ten days ; in the other 1·0 gm. was given by constant drip over a period of five days. Very similar results are reported from the Pacific area by Galliard and Mille (1949) and

Brygoo (1949). Of ninety-six patients treated by the former, eighty-four were apparently cured and twelve greatly improved. A case of lymph-scrotum was also cured. Hetrazan has no effect on *Achantocheilonema perstans*, either larvæ or adults.

In the streptococcal infections which complicate filarial disease due to *Wuchereria* sp., prontosil and proseptasine are of value in treating the lymphangitis. They have been used in doses of 1·5 gm. daily for six days with success by Floch (1936), Advier (1937), Berny and Gippet (1937) and Chabeuf (1938). Earle (1941) found sulphapyridine of value in the treatment of lymphangitis, funiculitis, mastitis and tenosynovitis but valueless in filarial myositis. There is no evidence that the sulphonamides have any specific action on the filariæ themselves, and Hawking (1940) did not observe any benefit from the injection of either 4, 4′-diamino diphenyl sulphone or prontosil soluble intramuscularly or sulphanilamide orally.

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PHENOTHIAZINE

Phenothiazine, or, as it is more correctly termed, thiodiphenylamine, was first synthesised by Bernthsen (1885). Since then it has been much used in the dye industry, as it represents the parent substance of the thiazine dyes. It was not, however, until 1934 that it was first employed biologically, when Campbell and his colleagues found that it had a lethal action on the larvæ of culicine mosquitoes. Other investigations on its activities showed that it could be used in controlling the breeding of hornfly larvæ in the fæces of cattle (Knipling, 1938).

In 1938, Harwood, Jerstad and Swanson discovered that phenothiazine had an action on ascarids and nodular worms in swine. Since then it has been tested as an anthelminthic in man and in the majority of domestic animals and birds. At first it was thought to be practically non-toxic, but later observers have shown that its administration may be followed by symptoms of poisoning, affecting more especially the hæmopoietic system.

Small repeated doses, too small to cure, will nevertheless control the degree of worm infestation and reduce the degree of egg production. Such action is not seen with other anthelminthics.

Phenothiazine, when pure, is a pale yellow crystalline powder, almost insoluble in water, with a faint but bitter taste. As commonly employed, however, it is a soft bulky powder of a greyish or even bluish-green colour. It is wettable only with difficulty. The solid melts at or slightly above 181° C. As a 2 per cent. solution in acetone it gives a clear bright solution not darker than pale olive green and with not more than a trace of insoluble matter. If kept dry, phenothiazine is relatively stable, but if moist it undergoes spontaneous oxidation, in most cases to either thionol or phenothiazone, both of which are red dyes. The immediate precursors of these red dyes are colourless leuco compounds, the systems leuco compound-dye being reversible, since it is the oxidation-reduction potential of the system which determines whether it is the leuco compound or the dye which is present at any particular moment. If the oxygen pressure in the system is below a certain limit, or if reducing agents are present, the leuco form exists : if oxidising agents are present and the oxygen pressure is above a certain limit, the dye is formed.

DeEds, Eddy and Thomas (1938) studied the excretion of phenothiazine after oral administration to rats, rabbits and man. When the urine of an animal dosed with phenothiazine is allowed to stand it develops a red colour, showing that phenothiazine is excreted, in part at least, as the leuco form of one of its red dye derivatives. Actually phenothiazine appears in the urine in two forms, as a water-soluble conjugated derivative (phenothiazine and an unknown), and secondly as the system leucothionol-thionol. The conjugated derivative can be broken down by treatment with strong hydrochloric acid in the cold when the resulting precipitate can be purified by extraction with alcohol. The purified product has a melting point of approximately 180° C., which is also the melting point of phenothiazine. The leucothionol-thionol system is identified by its potentiometric characteristics. These results were confirmed in sheep by Lipson (1940). In addition to the identification of phenothiazine by its melting point, he extracted the filtrate with chloroform and separated the constituents of the extract by selective adsorption on alumina. Thionol, identified by its absorption spectrum, was adsorbed on alumina, but phenothiazone, identified by its melting point, was not. It was therefore concluded that the colour developing in sheep's urine was due both to thionol and phenothiazone.

Collier (1940), on the other hand, basing his work on the oxidation to the coloured derivative and its colorimetric estimation (Eddy and DeEds, 1937), believes that the water-soluble conjugate is formed from leucophenothiazone and a potassium salt, probably potassium sulphate. The same conjugate is found in blood and milk. Using Collier's modification of the colorimetric method, Swales and Collier (1940) estimated that in sheep it was possible to recover up to 90 per cent. of the phenothiazine administered, equally divided between the fæces and the urine. Phenothiazine began to appear in the fæces about eight hours after dosing but traces of it were still demonstrable in the stomach and intestine four days later. The derivative which was believed to be conjugated leucophenothiazone was found in the blood stream, in measurable amounts, in from one to seventy-two hours after dosing and in the urine in from 30 minutes to 102 hours. The maximum concentrations in blood and urine were observed about six hours after a dose. Since blood and urine concentrations are closely parallel, it follows that excretion almost certainly closely follows absorption. The fate of phenothiazine appears to differ in the various species of domestic animals. According to Collier, Allen and Swales (1943), in sheep and horses potassium leucophenothiazone sulphate is found in the urine. In the dog, phenothiazone is present, while in the pig there is no conjugated phenothiazone but thionol and a sulphonium salt. In rabbits Benham (1945) has found that glucuronic acid conjugates of leucophenothiazone and possibly of leucothionol are present in the urine.

In the urine, DeEds *et al.* (1939) found that phenothiazine acts as an antiseptic. In the same way Swales and Collier (1940) noted that in lactating ewes, given phenothiazine by mouth, milk did not decompose for several days when kept in a warm room: the milk, however, turned pink after some hours' exposure to light and air. Lipson and Gordon (1940) obtained very similar results in sheep, for in twelve experimental animals an average of 32 per cent. of the oral dose was recovered in the fæces, in three instances more than 50 per cent.

The significance of these findings in relation to anthelminthic activity has been discussed by Davey and Innes (1942) with special reference to the question of whether phenothiazine is the actual anthelminthic. On the one hand, Gordon and Lipson (1940) found that phenothiazone had no anthelminthic action in sheep, but Collier (1940) has suggested that leucophenothiazone, leucothionol or thionol may be the active substance because these substances strongly inhibit certain enzymes, mammalian catalase and the cytochrome oxidase system, whereas phenothiazine itself has no action on either of these systems. Swales and Collier (1940), however, detected a conjugate of leucophenothiazone in the urine within half an hour of administration. As phenothiazine is itself so insoluble, part of it must undergo very rapid oxidation and conjugation in the upper part of the digestive tract: the conjugated leucophenothiazone might therefore be so rapidly removed that it could hardly have much anthelminthic action.

DeEds and Thomas (1941) believed that the anthelminthic action of phenothiazine was due to thionol excreted into the intestine with the bile and aided by the bile. This view was based on the finding that in man, the rabbit, dog, and sheep (Davey, 1942) phenothiazine is in part excreted in the bile in the form of phenothiazine, leucothionol, and thionol. Phenothiazine itself has little or no action *in vitro* on pig *Ascaris*, while thionol alone has only a slight action; this action is increased by bile. However, as Davey and Innes (1942) point out, thionol in sheep is inactive against *Trichostrongylus* sp. and *Hæmonchus contortus*, while phenothiazine is most active against worms whose habitat is in the stomach, cæcum and colon. In the stomach it cannot be aided by bile salts and thionol is not present; in the cæcum the bile salts are absent, since they have been largely absorbed.

Davey (1942) found that, unlike most efficient anthelminthics such as carbon tetrachloride, tetrachloroethylene and hexylresorcinol, phenothiazine does not act through the cuticle but only after being taken up through the mouth of the nematode into its alimentary tract, a fact which explains not only the large anthelminthic dose required but also the importance of a finely particulate phenothiazine preparation.

At present, however, there is no satisfactory explanation why phenothiazine should be particularly effective against " stomach worms " of ruminants and worms from the cæcum and colon, except *Trichuris*, and only partially effective against most intestinal worms.

Administration

Since the solubility of pure phenothiazine in water is very low—it has been estimated at 1 : 800,000—and in addition it has a pronounced " unwettability," it cannot be given in measurable amounts in aqueous solution nor can it be suspended in water without the addition of a wetting or dispersing agent. Usually sodium cetyl sulphate is used as a wetting agent, generally in a proportion of 1·25 per cent. The pure or " unconditioned " powder must therefore be given to animals either in gelatin capsules or mixed with the food. The majority of animals eat the powder readily, but there is a danger that some animals may receive excessive doses. If tablets are used, a binding agent must be mixed with the drug, but it must be such as to make it possible for the tablet to break up easily in the alimentary canal. A dispersing agent should, according to Davey and Innes (1942), also be incorporated. Swales (1940c) has produced such a tablet: its composition is: commercial phenothiazine (95 per cent. pure) 80 parts; starch 8 parts; sodium bicarbonate 5 parts; tartaric acid 4 parts; sodium choleate 2 parts. Phenothiazine has a constipating action which phenolphthalein is thought to counteract, but the laxative effect of phenolphthalein may also decrease the amount of phenothiazine absorbed. The drug has been used in the form of a relatively stable suspension containing approximately 40 per cent. (weight/volume) of phenothiazine, as a soya bean pellet, containing 27 gm. of the drug per lb., as a bolus or drench, and as a commercial aqueous suspension of phenothiazine, 1 fluid oz. containing 12 gm. of the drug. In addition, for sheep, perhaps the most satisfactory method is a salt-lick consisting of a mixture of phenothiazine and salt, usually 1 : 10 to 1 : 15. Sheep consume about 0·66 lb. of phenothiazine a year by this method, being thus protected from acquiring infection.

There seems little to choose between different methods of administration, provided the unconditioned drug is always finely powdered before use. Thorning, Sampson and Graham (1944), for instance, found no statistical difference between the effects of phenothiazine given to sheep as a capsule, bolus, drench or as a pellet with soya bean flour. Britton and Miller (1944) found a salt-lick of phenothiazine and salt 1 : 10 better than 1 : 15, and better for sheep than phenothiazine in capsules, pellets, and drenches. In the combined trials in Great Britain there was a suggestion, not verifiable statistically, that drenches may be slightly more effective than tablets against stomach worms only. The dangers attendant on mixing phenothiazine with the food of animals have already been mentioned: capsules are inconvenient because of the necessary bulk and the liquid suspension has the disadvantage that if any is spilled it will be converted into the oxidation products, thionol and phenothiazone, which in the case of sheep, stain the fleece red, a colour which cannot easily be removed. In Australia a non-automatic syringe is used to avoid staining (Council of Scientific and Industrial Research, 1945).

Attempts to increase the anthelminthic potency of phenothiazine by adding other substances have not been very successful. Prickly ash bark, for instance, is said to increase the insecticidal action of pyrethrum as a result of its content of ascarinin, sesamin, and sesame oil: added to phenothiazine there was no increased action against *Heterakis* and *Ascaridia* in fowls (Harwood and Guthrie, 1944b).

THE ACTION OF PHENOTHIAZINE ON THE WORMS OF DOMESTIC ANIMALS

Host.	Helminth.	Location.	Action.	Host.	Helminth.	Location.	Action.
Sheep	*Hæmonchus contortus.*	Stomach	+ + + +	Cattle	*Œsophagostomum* spp.	Large intestine.	+ + +
	Œsophagostomum columbianum.	Large intestine.	+ + + +		*Trichostrongylus* spp.	Small intestine.	+ +
	Ostertagia spp.	Stomach and small intestine.	+ + +		*Bunostomum trigonocephalum.*	Intestines.	+ +
	Trichostrongylus axei.	Stomach	+ + +		*Chabertia ovina.*	Large intestine.	±
	Bunostomum trigonocephalum.	Large intestine.	+ + +		*Cooperia* spp.	Small intestine.	Unknown
	Trichostrongylus colubriformis and *T. vitrinus.*	Small intestine.	+ +		*Skrjabinema ovis.*	Cæcum.	Unknown
	Cooperia spp.	Small intestine.	+ +		*Strongyloides papillosus.*	Small intestine.	Unknown
	Nematodirus spp.	Small intestine.	+ + or ±		*Hæmonchus contortus.*	Stomach and small intestine.	+ + + +
	Chabertia ovina	Large intestine.	±		*Œsophagostomum radiatum.*	Large intestine.	+ + + +
	Strongyloides papillosus.	Small intestine.	±		*Ostertagia* spp.	Stomach and small intestine.	+ + + +
	Trichuris ovis	Cæcum.	0		*Trichostrongylus axei.*	Stomach.	+ + + +

	viviparus.		
	Muellerius capillaris	Lung.	Unknown
Pigs	*Œsophagostomum dentatum.*	Large intestine.	+ + + +
	Ascaris lumbricoides.	Small intestine.	+ +
	Hyostronglyus rubidus.	Stomach.	+
	Ascarops sp.	Stomach.	+
	Physocephalus sp.	Stomach.	+
	Crassisoma urosabulatum.	Small intestine.	0
	Macracanthorhynchus hirudinaceus.	Small intestine.	0
	Trichuris trichiura	Large intestine.	0
	Stephanurus dentatus.	Kidney.	0
	Metastrongylus sp.	Lung.	0
Goats	*Hæmonchus contortus.*	Stomach.	+ + + +
	Trichostrongylus axei.	Stomach.	+ + +
	Ostertagia spp.	Stomach.	+ + +

	phlebotomum.	intestine.	(large doses)
	Cooperia spp.	Small intestine.	+ + or ±
	Trichuris sp.	Cæcum.	+ or 0
	Strongyloides papillosus.	Small or large intestine.	+ or 0
	Nematodirus spathiger.	Small intestine.	+ or 0
	Capillaria sp.	Small or large intestine.	+ or 0
	Neoascaris vitulorum.	Small intestine.	Unknown
	Dictyocaulus viviparus.	Lung.	Unknown
Hen	*Heterakis gallinæ*	Cæcum.	+ + + +
	Ascaridia galli.	Small intestine.	+ +
	Capillaria spp.	Stomach and small intestine.	++++(?)
	Subulura brumpti	Cæcum.	+ +
	Acuaria (Cheilospirura) hamulosa.	Œsophagus.	0
	Hymenolepis carioca.	Large intestine.	0

+ + + + Excellent results. + + + Very good. + + Good. + Slight.
± Doubtful. 0 No action.

Anthelminthic Action

Sheep. Many millions of sheep have now been treated with phenothiazine in Great Britain, Australia, Canada, and the United States of America. It is generally agreed that total doses should not exceed from 20 to 40 gm. per animal. If smaller doses are given, the action on *Hæmonchus* and possibly on some other worms will be demonstrable but with less certainty. Gordon (1943), in Australia, found that when injected into the rumen of sheep, 4 to 6 gm. of phenothiazine was invariably effective, while doses of 5 gm. per 30 kgm. of body weight permanently reduced the egg count by 99·5 per cent. In older and heavier sheep a dose of 10 gm. was effective in removing *Hæmonchus contortus*, but double that dose was necessary against *Œsophagostomum columbianum* and *Trichostrongylus* spp.

While in adequate doses phenothiazine will remove all *Hæmonchus contortus*, its efficiency is somewhat less against *Trichostrongylus axei*, *Ostertagia circumcincta*, and *O. trifurcata*. It is thus very active against worms normally parasitic in the abomasum. Gordon (1943) found that phenothiazine had very little action on immature *T. colubriformis* even when the drug was given direct into the rumen or abomasum. Gordon (1940a) also showed that it will remove the immature stages of *H. contortus*, a point of importance since it renders unnecessary repeated treatment at short intervals.

It is highly effective in removing the nodular worm, *Œsophagostomum columbianun* from the colon.

Swales (1940c), Robertson (1941) and Peters and Leiper (1941) all obtained good results against *Chabertia*. In doses of approximately 20 gm. per animal phenothiazine had a good effect against *Bunostomum* (Habermann and Harwood 1940, Swales 1940b).

Phenothiazine is less effective against intestinal worms, such as *Trichostrongylus* sp, *Cooperia* sp. and *Nematodirus spathiger*. Swales (1940c) and Taylor and Sanderson (1940) found an efficiency for these worms of 50 to 90 per cent., but others have failed to obtain such good results (Singer and Baker, 1940, and Stewart and Crofton, 1941). Seghetti and Marsh (1947), however, found a dose of 37·5 gm. per 95 lbs. suitable for controlling *T. vitrinus* and *T. colubriformis* in lambs.

Neither the tape worm *Moniezia expansa* nor *Tricocephalus ovis* appear to be susceptible to phenothiazine, and only Peters and Leiper (1941) have recorded any effect on *Strongyloides* sp.

These results have been confirmed by extensive trials carried out in Great Britain in 1942–43 in which flocks in different parts of the country took part, the efficacy of the drug being estimated by egg counts, worm counts, and body weight (Imp. Agric. Bur., 1943).

Where comparative trials of phenothiazine and the usual copper sulphate and nicotine sulphate mixture have been carried out they have almost shown that phenothiazine is to be preferred (McEwen 1940, Singer and Baker 1940, Boddie *et al.*, 1941, Stewart and Crofton, 1941, and Shorb, Habermann and Heemstra, 1941). Willman and Baker (1943) found phenothiazine superior for *Ostertagia circumcincta* and *Nematodirus spathiger* but only equal to the copper sulphate, nicotine-sulphate mixture for *Hæmonchus contortus.* Singer and Baker (1940) and Britton and Miller (1944) found phenothiazine superior to carbon tetrachloroethylene.

The question how far phenothiazine can be used prophylactically against nematodes in sheep has received attention. Swales (1940b), in Canada, advocated a single large dose of 30 to 40 gm. in the spring. Others have found that lambs may be given a salt-lick for considerable periods from birth onwards. Rotation of grazing, combined with drug treatment, can be almost completely effective in prophylaxis.

Peterson *et al.* (1944) showed that the best method of keeping a flock relatively free from worms is to give the ewes a therapeutic dose before they are put out to pasture. Both the ewes and their lambs are then allowed access to a phenothiazine salt-lick for the whole of the summer. Such a scheme is satisfactory only if the pasture has not been occupied for at least two months previously by infected sheep, and also if the treated flock is so maintained that there is no contact with other sheep.

Thorp *et al.* (1944) recommended drenching the whole flock with 12·5 gm. of phenothiazine ; this drench is repeated in spring one to two weeks after the ewes have lambed and before the flock is placed on a 1 : 9 phenothiazine salt mixture. Thorp *et al.* (1945) reported the feeding of a 1 : 9, phenothiazine-salt mixture to twenty ewes for three years without any toxic effects and a resulting very low average egg count. Although such treatment is able to keep the parasite load from becoming heavy it will not remove an initial heavy load (Turk, 1945). Treatment of lambs with phenothiazine or with a salt-lick containing phenothiazine reduces pasture larval counts (Harbour *et al.*, 1946). The strength of these mixtures varies from 9 to 1 to 14 to 1 (Hawkins *et al.*, 1944 ; Seghetti and Marsh, 1945), but according to Pollard *et al.* (1949) monthly dosage with 20 gm. is more effective than a 1 in 10 phenothiazine-salt mixture. Page (1949) finds that 0·5 gm. daily for fourteen days before and seventy days after infection protects non-immune lambs from a single massive dose of larvæ.

Goats. Comparatively few tests have been made in goats, but Taylor and Sanderson (1940) record results similar to those in sheep. Turk (1945) found no activity against *Trichostrongylus* in goats.

Cattle. As in sheep, the activity of phenothiazine is most marked on nematodes living in the abomasum and colon. Swanson, Porter and Connelly (1940), Taylor and Sanderson (1940), Swanson and Carlisle (1941), Porter *et al.* (1941) and Porter (1941) reported the effectiveness of the drug against *Hæmonchus*, *Trichostrongylus axei* and *Œsophagostomum* but found that it had little or no action against other helminths, though Roberts (1941) recorded some effect against *Cooperia.* In Nigerian zebu cattle, weighing 120 to 130 kgm., Sprent (1946) found phenothiazine of value in doses of 30 gm. for *Hæmonchus contortus* and *Œsophagostomum radiatum* but ineffective against *Bunostomum phlebotomum* for which, according to Swanson, Porter and Connelly (1940), doses of 0·7 to 1·1 gm. per kgm. are required.

There is evidence that the tolerance of cattle for phenothiazine is less than that of sheep ; the dose should be kept as low as possible, particularly in calves : 0·2 gm. per lb. of body weight is probably effective for most worms in cattle, but a total dose of 40 to 100 gm. was not toxic even for weak calves (Bruford and Fincham, 1945).

Pigs. Critical anthelminthic tests were carried out by Swanson, Harwood and Connelly (1940) to confirm the earlier work of Harwood, Jerstad and Swanson (1938).

In doses of approximately 0·2 gm. per lb. of body weight more than 90 per cent. of *Œsophagostomum* were expelled and at least

50 per cent. of *Ascaris*. On the other hand, there was no action on the stomach worms *Ascarops*, *Physocephalus* and *Hyostrongylus*, or against the hookworm *Crassisoma*, the thorny-headed worm *Mœracanthorhynchus*, and the whipworm *Trichuris*.

For *Œsophagostomum* doses of 0·25 gm. per lb. of body weight are recommended as higher doses of 0·8 gm. per lb. of body weight are liable to cause toxic reactions.

Enzie *et al.* (1945) compared phenothiazine with oil of chenopodium and sodium fluoride in swine. Phenothiazine given in the food to the extent of 0·2 gm. per lb. of body weight removed less than 1 per cent. of ascarids while sodium fluoride removed 100 per cent. Phenothiazine removed *Œsophagostomum*, for which oil of chenopodium was ineffective (Gordon, 1940b).

Horses. It is now generally agreed that doses should not exceed 30 gm. per 1,000 lb. of body weight for a full-grown animal and 15 to 29 gm. for a foal, otherwise symptoms of poisoning may occur. Horses should be carefully prepared by giving several bran mashes before treatment (Britton, 1944). Phenothiazine removes practically all Sclerostome and Cylicostome worms from the large intestine. Its efficiency against other nematodes is somewhat uncertain. Thus Howell and Britton (1940) found phenothiazine effective against *Trichostrongylus axei* and the pin worm *Oxyuris equi* but with no action against *Ascaris equorum*. On the other hand, Taylor and Sanderson (1940) found that *Ascaris* was satisfactorily expelled but that *T. axei* was not attacked, while Habermann *et al.* (1941) found a 72 per cent. efficiency against *Ascaris* but no action against *Oxyuris*.

Vianello (1942) obtained 90 to 100 per cent. efficiency against strongyles and 60 to 70 per cent. against oxyuris. Wetzel and Elksnitis (1943) likewise found the drug ineffective against *Oxyuris equi*.

Jardine and Knight (1942) found that *Strongyles* were removed from adult horses and *T. axei* from colts, but *Parascaris equorum* and *Gastrophilus* spp. were unaffected. Gordon and Whitten (1940) compared phenothiazine with other drugs for trichostrongylosylosis in horses.

Phenothiazine apparently has no action against bots, but Boley *et al.* (1941) demonstrated that 40 gm. of phenothiazine and

24 ml. carbon bisulphide, following a thirty-six hours' fast, removed *Ascarids* and *Strongyles* together with a large number of bots.

Small repeated doses of phenothiazine have been given to four horses in their food by Foster and Habermann (1944) ; 5 gm. was given per week either in a single dose or in repeated small doses till a total of 100 gm. had been administered to three horses and 70 gm. to the other. After a fortnight there was a reduction in the egg count in the fæces, the diminution in the count continuing so long as the drug was administered. Schmid (1944) gave 10 gm. daily for three to six days, but young horses aged six months to two years received 5 gm. for from four to six days. Elimination of worms begins twenty-four hours after the commencement of treatment and ceases after four days ; the peak takes place in from forty-eight to seventy-two hours (Velu and Train, 1943). Constipation may occur but it is not proportional to the dose.

Dogs. So far efforts to use phenothiazine as an anthelminthic in dogs have not been successful. It appears to be valueless against *Ascarids* (Montgomerie, 1940), hookworms, *Trichuris*, and the tape worm *Dipylidium.*

Steyn (1945) gave the drug intratracheally to dogs with lung worms, *Filaroides osleri*, but without effect.

Elephants. Cylicostome worms *Equinurbia sipunculiformis* and *Murshidia falcifera* were expelled by four daily doses of 10 gm.

Poultry. Phenothiazine has been given in doses of 0·05 to 0·5 gm. : the larger dose is effective in eliminating *Heterakis* but is less active against *Ascaridia.* In turkeys, Harwood and Stunz (1945) reported that phenothiazine was 95·9 and 99·7 per cent. effective against *Heterakis gallinæ* and 42·9 and 69·6 per cent. effective against *Ascaridia dissimilis.* In pigeons, Guilhon (1945) found phenothiazine highly effective against *Ascaridia columbæ* but less active against *Capillaria* sp. There was no action on cestodes (Roberts, 1940), the effect on *Davainea proglottina* claimed by Guilhon (1945) not having been confirmed. As " blackhead disease " of turkeys is transmitted from bird to bird by the eggs of *Heterakis*, Davey and Innes (1942) recommend phenothiazine for the control of " blackhead disease." Wehr and Olivier (1946), however, found that the addition of 1 or 2 per cent. of phenothiazine to the mash for four to six weeks did not affect the incidence of blackhead nor did it prevent infection with *Heterakis:* it did, however, result in expulsion of the worms before or soon after they reached maturity. Egg laying is not inhibited by dosing with phenothiazine (Temperton and Dudley, 1945), 1·5 or 4·4 gm. per month having been given to birds.

McCulloch and Nicholson (1940) prefer giving phenothiazine in capsules to mixing it with food. Harwood and Guthrie (1944a) found that coarse phenothiazine and finely divided " micronised " phenothiazine were equally effective, while no difference could be detected between a non-wettable and a wettable preparation containing gum karaya and algin. For the treatment of poultry, Harwood and Guthrie recommend tablets weighing 1·33 ± 0·29 gm. and containing 1 part of phenothiazine and 2 parts of nicotine bentonite. The latter is more effective against *Ascaridia galli*, the former against *Heterakis gallinæ.*

Phenothiazine has no effect on the development of the larvæ of *Trichinella spiralis* in the muscles of rats (McNaught, Beard and DeEds, 1939), but the oral administration to rats of 2-methyl-1, 4-naphthoquinone and 2-hydroxy-3-piperidinomethyl-1, 4-naphthoquinone is said to reduce significantly the number of adult worms (Oliver-González and Bueding, 1948).

A gorilla was treated by Manson-Bahr (1940) with doses of 0·3 gm. of phenothiazine for three days; eighteen *Œsophagostomum* worms were passed in a dead or dying condition but no ancylostomes were expelled.

Phenothiazine in Man

The first observations on the action of phenothiazine on human helminths were made by Manson-Bahr (1940), who gave it to three patients with *Ancylostoma duodenale*, nine with *Ascaris lumbricoides* and nine with *Enterobius vermicularis.* The hookworms were entirely unaffected, even with doses as large as 30 to 40 gm. In patients with round worms the effects were very variable: all round worms were expelled from two patients given 8 gm. daily for three days, followed by a dose of Glauber's salt, but from seven other patients, given doses of 16 to 48 gm., only one round worm was expelled.

All the patients infected with *Enterobius* were cured, but the doses given were far higher than would now be considered safe—2 gm. per day for seven days for children under the age of eight years, 1 gm. per day for seven days for children under four years, and 8 gm. per day for five days for adults. No toxic results were noted. Kuitunen-Ekbaum (1941) treated eighty-nine children and nine adults for enterobiasis. The drug was given to children in cereals or porridge, and to adults either suspended in water or in capsules: no purge was given. The dosage was as follows: 8 gm. for those aged nine years and over, 6 gm. for those aged six to eight years and 4 to 5 gm. for those aged two to five years. The dosage was spread over four to six consecutive days. While the youngest age group had about the same dose as that recommended by Manson-Bahr, the other patients had only about half his recommended dose. Of the eighty-nine children and nine adults treated, seventy-six children and eight adults were cured by a single course, and the remainder, except for one child, all responded to a second course. No toxic effects were observed. Later observers have not been so fortunate. Hubble (1941), for instance, observed three cases of acute hæmolytic anæmia and toxic hepatitis among some thirty patients treated with phenothiazine for *Enterobius* infections, while in 1942 the death of a girl six years of age occurred after the administration of phenothiazine. Instead of taking 2 gm. a day, as had been advised, the child took only 1·5 gm. on the first day, 2 gm. on each of the next three days, and 1 gm. on the fifth day, a total of 8·5 gm. She developed slight icterus on the fifth day with headache; on the tenth day, when admitted to hospital, her temperature was 101° F. and her hæmoglobin was 26 per cent., red cells 1,250,000 per c.mm., leucocytes 16,850 per c.mm., normoblasts 1 per cent., reticulocytes 22·6 per cent. and platelets 236,000 per c.mm. She died some hours after being given a transfusion of whole blood.

Sisk (1943) found a total dosage of 40 gm. for adults too toxic: a total dose of 20 gm. in five days gave rise to toxic symptoms in only ten patients out of eighty-nine treated. In two, both members of the same family, there was severe nausea, headache, vomiting, pallor and headache, with anæmia, nucleated reds in the blood stream, hæmaturia, and leucocytosis. A dosage of 20 gm. cured all patients, but in view of its toxicity this was reduced to 12 gm. The curative results were not quite as good but no toxic reactions were seen.

Most (1943) prefers to give a dosage based on body weight, 300 mgm. per kgm. of body weight being given during three days.

Elliott (1943) did not observe any toxic reactions among seventy-five West African soldiers given 2 gm. three times a day for four or five days, the tablets being ground up to help absorption. Results of treatment were not outstanding, for of thirty-six infected with *Necator americanus* only twenty-six were cured, of fifteen with *Ascaris lumbricoides* only nine, of eight with *Trichuris trichiura* only four, and of eight with *Strongyloides stercoralis* only five.

Bercovitz *et al.* (1943) obtained equally unfavourable results. Of ten patients with *Enterobius vermicularis* only two were cured, of three with hookworms none, while two patients with *Ascaris*, nine with *Trichuris trichiura* and two with *Tænia saginata* all remained positive. Most (1943) and Sisk (1943) also agree that phenothiazine is useless against other human intestinal worms.

It should be emphasised that much of the evidence for the value of various drugs in *Enterobius* infestation in children is entirely unsatisfactory. The apparently good results obtained by many observers are due to the normal periodicity of the infection resulting from the life cycle of the parasite. Only by the most careful and strictly regulated use of control groups, treated and untreated, under otherwise identical conditions can there be gained information on which reliance can be placed. Even under hospital conditions satisfactory control is extremely difficult. The problem is further complicated by almost complete impossibility in practice of ensuring freedom from reinfection.

Guinea Worms. Working in West Africa, Elliot (1942) has attempted to treat guinea-worm infection with intramuscular injections of phenothiazine. The drug is suspended in warm olive oil and injected as near the course of the worm as possible. Two or three sites were injected with 0·5 to 1 gm. of phenothiazine, while treatment was repeated at weekly intervals: no toxic effects were encountered in twenty-three patients, even when

2 gm. was given weekly for four weeks. Attempts to repeat these results in other military hospitals in West Africa failed.

Dracontiasis presents a most difficult chemotherapeutic problem. One or more worms may be present in a single patient and many sites such as the scrotum are unsuitable for the injection of phenothiazine. In the hands of a skilled extracter there is little difference in the rate at which a live or a dead worm can be extracted. If anything, the risk of breaking a worm appears to be greater in the case of a dead than a living worm : if a dead worm remains *in situ* near a joint it may undergo calcification and act as a foreign body. Many other treatments have been tested on guinea worms, including intensive courses of ter- and quinquevalent antimony salts, but without significant results.

Stefanopoulo (1947) records that phenothiazine appears to decrease the œdematous calabar swellings due to *Loa loa* and to reduce the number of microfilariæ in the blood stream.

Toxicity

The question of the toxicity of phenothiazine has been very fully reviewed by Davey and Innes (1942) and Edwards (1947). In man the possibility of phenothiazine causing a hæmolytic anæmia was first reported by DeEds, Stockton and Thomas (1939), who found that in using phenothiazine as a urinary antiseptic three of their patients developed anæmia after receiving 19·9, 22·4 and 28·1 gm. orally over periods of eight, ten and sixty-seven days respectively. Reference has already been made to the cases described by Hubble (1941) and Humphreys (1942). In addition, Johnstone (1942) described a girl, aged seven, who was given 1 gm. of phenothiazine twice daily for five days. She then collapsed with rapid pulse, fever, icterus, and delirium ; on examination, the blood changes were those of an acute hæmolytic anæmia.

The blood changes have been studied in man by Bercovitz *et al.* (1943). Twenty-four patients were given 1·3 gm. three times daily for ten days, a total of 40 gm. No significant changes in the leucocytes were seen, five showed no change in hæmoglobin, nine had a decrease of less than 10 per cent. and nine a decrease of more than 10 per cent. ; one was not examined. Six patients showed no reduction in the number of red cells, eleven a decrease up to

500,000 per c.mm., four from 500,000 to 1,000,000 per c.mm., and three a decrease of over 1,000,000 per c.mm. Six showed slight albumin in the urine and one a considerable amount.

Another possible toxic reaction to phenothiazine is that noted by Newcomer (1937) and DeEds, Wilson and Thomas (1940) among men using a suspension of phenothiazine as a spray for controlling the codling moth. After working in the sun, some of the men complained of intense itching, irritation and reddening of the skin, somewhat resembling acute sunburn. These changes may be due to photosensitisation produced directly or indirectly by the presence of the oxidation-reduction system, leucothionol-thionol, in the tissues. The dermatitis in pigs reported by Rietz (1942) might also be the result of photosensitisation, but an attempt to produce photosensitisation in white-faced sheep with phenothiazine was unsuccessful (Swales, 1940a). It is to be noted that in all the cases where toxic reactions have occurred in man there has been repeated administration of phenothiazine. Davey and Innes (1942) have therefore suggested that efforts should be made in man to test the anthelminthic action of a single dose of phenothiazone.

On the other hand, Eddy, Cox and DeEds (1937) showed that rats could withstand a daily dosage up to 0·25 per cent. by weight of their diet for 295 days, while McCulloch and Seghetti (1942) reported a pig weighing 110 lb. which was given 30 gm. of phenothiazine daily for forty-two consecutive days, a total of 1,260 gm. Apart from a slight fall in hæmoglobin concentration there were no untoward effects.

Collier and Allen (1942b) failed to produce anæmia in rabbits and guinea-pigs with large and repeated doses of phenothiazine even when the diet was deficient in vitamins of the B complex. Similar results were obtained by Schnitzer *et al.* (1942). In mice, sixteen daily doses of 1·25 gm. per kgm. of body weight caused no anæmia and no clinical symptoms or pathological changes. In guinea-pigs, repeated daily doses of 2 gm. per kgm. of body weight caused loss of weight and occasionally loss of hair but no anæmia. In dogs, anæmia was more readily produced, but after stopping the drug the hæmoglobin rapidly increased.

The toxic effects of phenothiazine vary in different species of animals, but in some cases phenothiazine toxicity is used to explain deaths due to other causes.

Horses. The available information on the toxicity to horses has been fully reviewed by Taylor (1942).

Doses of 90 to 112 gm. are excessive, even in horses in good condition (Hatcher, 1941 ; Errington, 1941). Doses of 60 to 90 gm. may very occasionally cause fatalities (Wolfe and Dennis, 1941 ; Schmidt, Christian and Smotherman, 1941).

For horses that are in poor condition doses of 15 to 45 gm. can produce severe reactions (Fincher and Gibbons, 1941). Sometimes the recommended dose of 30 gm. may be toxic (Taylor, 1942), but only certain animals are affected. Britton (1944) believes that it is not phenothiazine but the oxidation products which are poisonous. Digestive disturbances and constipation, tending to reduce peristalsis, increase absorption of the oxidation products—hence the importance of preparatory bran mashes.

The signs and symptoms of poisoning are very characteristic : oligocythæmia, hæmoglobinuria, and albuminuria with icterus and enlargement of the heart, spleen and kidneys. Loss of appetite and fatigue are marked.

The whole problem of phenothiazine toxicity is still somewhat obscure and the minimal lethal dose is not yet known for any farm animal.

Sheep. After giving sheep 10 gm. of phenothiazine daily, Taylor and Sanderson (1940) found that two sheep began to show signs of intoxication after the first week, after having received 70 gm. of the drug. Holman and Pattison (1941) noted that the phenothiazine treatment of sheep increased the severity of the anæmia due to helminths, though the sheep grew normally.

McNally (1943) found that if boluses of 12·5 gm. of phenothiazine were given to lambs, more than once in two weeks, pathological changes occurred in the kidneys. With frequent doses polycythæmia was noted and, with boluses once or twice a week, agranulocytosis. Turk *et al.* (1946) gave ten times the usual dose without toxic reactions to the mouflon, *Ovis musimon*, the Barbary sheep, *Ammotragus lervia*, and the Himalayan tahr, *Hemitragus jemlahicus*.

The administration of phenothiazine to sheep and goats during the later stages of pregnancy may cause abortion (Warwick *et al.*, 1946). Twelve of twenty-three ewes in the last three weeks of gestation aborted after doses of 25 gm. Administration to pregnant ewes is safe, except in the last three to four weeks of pregnancy. It is important to note that in sheep given phenothiazine a false van den Bergh reaction may occur. Within forty-eight hours of administration a colour is produced in the serum on the addition of nitrite in the presence of acid. The colour is not due to azobilirubin as the protein-free alcoholic solution does not change to a blue colour on the addition of concentrated hydrochloric acid (Clare and Simpson, 1943).

Cattle. Both Taylor and Sanderson (1940) and Roberts (1941) believe that cattle are more sensitive than horses or sheep to the toxic action of phenothiazine. Roberts (1941) gave eight cattle doses varying from 0·16 to 0·8 gm. per lb. of body weight and found some toxic reaction in all. With the smaller doses there was transitory anorexia and constipation, with the larger doses severe abdominal pain and inco-ordination of the hind legs. Cauthen (1945) found no anæmia in calves after 36 to 52 gm. of phenothiazine but a dose of 250 gm. caused anorexia and inco-ordination: these symptoms disappeared after twenty-four hours. Wise *et al.* (1947) gave 125 gm., twice the maximum recommended dose, to cattle without toxic symptoms. There is no evidence that the milk contains toxic products. Whitten *et al.* (1946) described a keratitis probably due to photosensitisation.

Pigs. Lapage (1940) noted that after a single dose of 0·5 gm. per lb. of body weight some three-month-old pigs exhibited toxic symptoms with ataxia. Roberts (1941) also observed ataxia in pigs given doses of 0·8 gm. per lb. of body weight. Rietz (1942), in addition to the dermatitis already described, saw ataxia in four-week-old pigs given 5 gm. each of phenothiazine.

Dogs. An anæmia in dogs is produced by the administration of 5 gm. per kgm. for several days (McNally, 1943); this anæmia is not abolished by giving large doses of the vitamin B complex. Heinz bodies, small dense bodies attached to the leucocytes, are, however, abolished by administration of vitamin B complex (Collier and Mack, 1944). Stefanopoulo (1947) gave 8 gm. by mouth in four days to a dog weighing 10 kgm.; no ill effects were seen.

In a cat 0·4 gm. per kgm. of body weight caused a severe but not fatal reaction (Krupski and Leeman, 1943).

Fowls. White fowls may be stained pink or red by phenothiazine but the toxicity is very low. Harwood and Guthrie (1944a) found that as much as 8·8 gm. of phenothiazine and twice as much nicotine bentonite caused nothing except temporary loss of weight, probably due to nicotine.

The factors responsible for the toxicity of phenothiazine are as yet not fully understood. Different species vary in their susceptibility, sheep being probably the least and cattle, horses, and young human beings the most susceptible. Pigs are not as easily poisoned as was at first thought. Young animals are more susceptible than adults of the same species. Idiosyncrasy does not appear to be a satisfactory explanation for all cases of poisoning, for toxic manifestations tend to occur in groups. Possibly factors tending to cause intestinal stasis increase absorption and thus the liability to poisoning, but there is no reliable experimental or authoritative evidence to support this view.

Evidence on the hæmolytic action of phenothiazine in different species has been brought forward by Collier and Allen (1942a). No direct hæmolytic action on the red cells of horses or sheep was exerted by phenothiazone *in vitro*, but by adopting the technique described by Ponder (1941) they were able to show that there is an accelerating effect on hæmolysis produced in certain animal species by derivatives of phenothiazine. Ponder's method consists in determining the time required to hæmolyse 50 per cent. of washed red cells by a known amount of lysin, such as saponin or lysolecithin : then the time required is determined for a 50 per cent. hæmolysis with the same amount of lysin in the presence of the test substance.

Although much has been made of the toxic effects of phenothiazine it must be remembered that enormous numbers of animals have been treated without any evidence of poisoning.

In vitro the hæmolysis of horse erythrocytes by saponin or lysolecithin was powerfully accelerated by the presence of phenothiazine, thiazine S-methyl sulphonium perchlorate, and especially the urinary conjugate, potassium leucophenothiazone sulphate. In the presence of these substances sheep red cells are far less readily hæmolysed by saponin than are horse red cells; horse red cells are also readily lysed by 0·026 per cent. sodium chlorate, but this is not accelerated by phenothiazone. Phenothiazone and thiazine S-methyl sulphonium perchlorate are also powerful inhibitors of the enzyme chloesterinase (Collier and Allen, 1942b). In addition, various oxidation products of phenothiazine are able to inhibit catalase, cytochrome oxidase, and dehydrogenases, but whether this action plays any part in the toxicity of the drug is unknown (Collier, 1940; Collier and Allen, 1942c). In the plasma of horses there is apparently a sufficient concentration of potassium leucophenothiazone sulphate to produce a very considerable acceleration of saponin hæmolysis *in vitro*. While the conjugation of leucophenothiazone to the sulphate is a detoxifying mechanism it may, in the light of these experiments, actually intensify the hæmolytic effect of phenothiazine.

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FICIN OR LÈCHE DE HIGUÉRON

The use of the latex of figs against worms is by no means new, but within recent years considerable work has been carried out on purified preparations which appear to have a specific action on the whipworm *Trichuris trichiura L.* There is evidence that in the eighteenth century the latex of certain fig trees, mixed with water, was used in Cayenne, French Guiana (Thomen, 1939), as an anthelminthic. The first scientific account was given by Bajon (1770 and 1771), who asserts that the latex was prescribed on the advice of an African negress : there is thus uncertainty whether or not the treatment was introduced to South America from Central Africa ,where latex from the rubber tree has long been used (Mouchet and Hoebeke, 1934). Latices of many species of figs have been used medicinally in China (Dragendorff, 1898), Greece, Rome, and India (Ainslie, 1826). The first chemical studies on the latex of *Ficus doliaria* Mart. demonstrated a glucoside named doliarina and a proteolytic enzyme termed urostigma papayotin (Peckholt, 1861). Rational therapeutic use of the latex as an anthelminthic was begun by Wucherer (1866). Later, a powder containing crystals of doliarina, iron, and an aromatic vegetable substance was employed in Turin by Bozzolo (1880–81) against ancylostomes, and for some years various preparations continued in use as anthelminthics against ancyclostomes and ascaris.

In 1911 Calle and Uribe reported on the use of lèche de higuéron in infections with whipworms, results which were confirmed by Paez (1914–15), Schapiro (1925), Hall (1923) and Caldwell and Caldwell (1929). These observers used the latex of *Ficus laurifolia*, as did Hall and Augustine (1929) who, in experiments on Colombian soldiers, found that the drug had only a limited usefulness in combating hookworm infection.

The digestive action of ficus was demonstrated by Moncorvo (1881); later Robbins (1930) showed that the anthelminthic substance in the crude sap of *Ficus laurifolia* was an enzyme which gave all the colour tests for proteins. The latex contained 25 per cent. by weight of this crude substance, which could be precipitated by magnesium sulphate or mercuric chloride. After re-dissolving and precipitating several times, a light yellowish powder was obtained to which the name ficin was given. In concentrations of 0·1 to 0·2 per cent. in Ringer's solution ficin is capable of digesting living ascaris in about two hours. The enzyme is more like papain than pepsin; it is inactivated at a pH below 4 and destroyed by a temperature of 75° C. or above. Asenjo (1939) found that an exposure for five minutes at a temperature of 85° to 90° C. was sufficient for complete inactivation. Robbins (1935) showed that the optimum hydrogen-ion concentration for gelatin proteolysis by ficin is pH 5. While a semi-refined crystalloid has been prepared, Walti (1938) obtained the enzyme in a crystalline form, when it is said to have a sulphur content of 1·6 per cent. The activity of the crystalline preparation is destroyed by hydrogen peroxide and iodine.

The exact distribution of ficin in various species of ficus has been investigated by Robbins and Lamson (1934), who studied the sap from sixteen different species. In only two of these, *Ficus carica* from Alabama and *F. glabrata* from South America, was the concentration in a 2 per cent. solution of sap sufficiently great to digest ascaris ; *F. nitida* had some action. The latex of *F. laurifolia* and *F. doliaria* is active as is that of *F. pumila* (Asenjo, 1939). There is some evidence to show that the ficin content varies seasonally, for Robbins (1935) found that in the case of *F. carica* the amount present is appreciably less in summer than in winter.

Some of the properties of ficin have been discussed by Andrews and Cornatzer (1942), who showed that the enzyme is not destroyed by the digestive activities of the dog, since it can be extracted from the dog's fæces with little loss, a point of some importance for chemotherapy as the normal habitat of *Trichuris* is the cæcum. Molitor *et al.* (1941), on the basis of sensitisation to anaphylactic shock, found no evidence of absorption of ficin from the intestinal tract. By mouth the LD 50 for rats and mice is about 10 gm. per kgm. ; following intravenous injection the toxicity was high, 50 to 100 mgm. per kgm. The toxicity of a given dose was reduced by subdivision into smaller doses given repeatedly. Oral administration over a period did not alter liver or kidney function tests. Intravenous injection, however, reduced the erythrocyte count and prolonged the coagulation time of the blood.

Faust and Thomen (1941) conducted *in vitro* tests on the whipworm of the dog with (1) the crude latex refrigerated and chemically preserved by the addition of from 0·1 to 1 per cent. sodium benzoate, (2) the amorphous ficin, and (3) the semi-refined crystalloid, sealed *in vacuo*. The most efficient preparation was the unpreserved and refrigerated crude latex ; the preserved latex was a little less active. A 10 per cent. solution of the crystalloid compared favourably with the crude latex when freshly opened, but rapidly deteriorated when exposed to air. The amorphous ficin, while relatively stable, was only about half as efficient as the fresh crystalloid. If ficin is to act efficiently it is important first to clear the large bowel of fæces (Faust and Thomen, 1941).

Ficin appears to act on the whipworm by direct digestion of the cuticle and not after absorption into its alimentary canal. Though some observers report excellent results with ficin (Brown, 1934), others have failed to expel any appreciable number of whipworms. Einhorn and Miller (1946), for instance, found latex either fresh or preserved with sodium benzoate quite ineffective when given by mouth. In two children, however, after hexylresorcinol, medicinal gentian violet, and eight courses by mouth of from 30 to 90 ml. of latex had failed to bring about a cure, five enemata of 90 ml. of fresh latex, given on alternate days, removed from 50 to 200 whipworms at a time. Differing results may be due either to differences in the enzyme content of the latex used or to the amount of fæces present in the cæcum.

For those who have to deal with Californian sea lions, *Zalophus californianus*, it may be of interest to note that the nematode stomach worms of the sub-family *Anisakinæ*, by which these sea lions are infested, are readily destroyed by crude latex preserved by freezing; commercial preparations preserved with sodium benzoate are less effective (Herman, 1942).

Toxic symptoms may occur in man (Montoya, 1919–20); colic, nausea, vomiting, muscular cramps, delirium, syncope, urticaria, rectal and vesical spasm, and partial suppression of urine have all been noted. Lamson, Brown and Ward (1935) found that in animals the toxicity of ficin was greatly increased if it was given two to three hours after an oral dose of hexylresorcinol, which sets up a temporary irritation. Toxicity in man may therefore be greater if there is previous damage to the intestinal mucosa. In animals, sub-lethal doses cause vomiting, bloody diarrhœa, and general prostration. At necropsy there is severe irritation of the gastro-intestinal tract with inflammatory changes or even mucosal erosion. Parenteral injection of ficin causes severe tissue injury (Molitor *et al.*, 1941). Such reactions, however, are rare and, provided that active preparations are used, ficin is probably a non-toxic and comparatively efficient remedy for whipworms.

Ficus latices are official in the pharmacopœias of France, Spain, and Mexico.

The comparative effects of ficin and emetine hydrochloride given by mouth were compared by Burrows *et al.* (1947), who found emetine in enteric-sealed tablets more efficient. Each tablet contained 0·3 gr. (18 mgm.), and three tablets were given daily for twelve days or six tablets daily for six days. In some cases nine tablets were given daily for one or two days, but results were less satisfactory than when smaller doses were administered for longer. In all, twenty-three patients were treated : eleven lost all adult worms while eighty-eight per cent. of the adult worms were lost by the group. Nausea and vomiting occurred in a few patients, diarrhœa with blood and mucus in all. Emetine hydrochloride was less variable in its action than lèche de higuéron and less drastic in its action on the patient than ficin in large doses. Emetine also caused elimination of some *Enterobius*, *Ascaris* and *Necator.*

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OTHER ANTHELMINTHIC DRUGS

(1) Dyes as Anthelminthics

In view of the success of phenothiazine, other dye-stuffs have been tested for anthelminthic action. Deschiens (1944a and b), Deschiens and Lamy (1944) and Pautrizel (1945) studied certain derivatives of triphenylmethane, including acid and basic fuchsin, malachite green, methyl green, and brilliant green. Anthelminthic potency appears to be related to the number of amino groups in the molecule, activity increasing with increase in the number.

It was found that all the dyes tested had some action on oxyurids of mice, *Syphacia obvelata* and *Aspiculuris tetraptera*, when mice of 20 gm. body weight were given 0·75 ml. of 1 in 2,000 aqueous solution for from eight to ten days. In addition to these nematodes, human and rabbit threadworms (*Enterobius vermicularis* and *Passalurus ambiguus*) and dog and human round worms *Toxocara canis* and *Ascaris lumbricoides* were susceptible. Basic fuchsin was highly effective against ascaris in dogs in doses of 0·02 to 0·04 gm. per kgm. of body weight for ten days, and also in doses of 0·03 gm. per kgm. against *Dipylidium caninum.* Malachite green was specially active against oxyurids of the rabbit. The toxicity of these dyes for man is low, although occasionally they may produce nausea and vomiting.

Gentian violet has also received considerable attention as an anthelminthic since it was first employed orally by Faust and Ke-fang (1926) in the treatment of infestation by *Clonorchis sinensis.* For medicinal purposes it should be free from dextrin. As usually supplied, gentian violet is described as a mixture of tetra-, penta- and hexa-methyl*para*rosaniline chlorides. As these three compounds show no appreciable difference in therapeutic effect, and as the hexa-compound, crystal violet, is most readily obtainable in the pure state, the latter is usually employed medicinally.

Gentian violet appears to be of particular use in cases of *Enterobius* infection in man (Wright *et al.*, 1938), (Wright and Brady, 1940) ; it is also of value against animal threadworms. The dosage of the drug, in enteric-coated tablets is, for a human adult, 64 mgm. three times a day before meals for sixteen consecutive days, and for children 10 mgm. a day for each year of apparent age, the total daily amount being divided into three doses. The enteric coating involves the use of phenyl salicylate with some keratin. D'Antoni and Sawitz (1940) prefer seal-ins or enseals tablets : two tablets of 0·03 gm. are given three times a day for eight days, followed by a week's rest, then a further course for eight days. Piccioli (1948) gave thirty-two children keratin-coated capsules containing 10 mgm. for each year of age. Two courses, each of five days' duration, with five days' rest cured all the children.

Transient minor disturbances, such as loss of appetite, nausea, vomiting, abdominal pain, and diarrhœa may occur, but since they are mainly due to irritation of the gastric mucosa by the drug they may be avoided by the use of enteric-coated capsules. Otherwise there are no toxic reactions, but Wright and Brady (1940) believe that concomitant infestation with *Ascaris lumbricoides*, alcoholism, and cardiac, renal and hepatic damage are contra-indications.

For rabbits the minimal lethal dose by mouth is 22 mgm. per kgm. of body weight for six days. A dog survived a dose of 35·4 mgm. per kgm. of body weight given for eighteen days.

In human infections with *Strongyloides stercoralis*, gentian violet is also apparently of considerable value (Sioe, 1928; de Langen, 1928, and Faust, 1936), while Maplestone and Mukerji (1939) regard gentian violet as the best remedy for *Hymenolepis nana* infection, 1 gr. (0·06 gm.) being given three times a day for one week or several courses for three days with an interval of a week between courses. Gentian violet is of no value in ascariasis, ancylostomiasis, and trichocephaliasis (Einhorn and Miller, 1946).

Acridine dyes have also been employed. In 1933 Kutschinsky claimed to have cured mice infected with the dwarf tapeworm *Hymenolepis fraterna* by administration of acetarsol. Maplestone and Mukerji (1939), however, found acetarsol useless. Later, Culbertson (1940) used mepacrine in the treatment of experimental infections in mice; oral administration of 0·01 gm. on two successive days or somewhat smaller doses for prolonged periods were found to kill the worms: subcurative doses suppressed egg-laying so long as the drug was present in the intestine. Culbertson and Greenfield (1941) also investigated the effect of mepacrine on *Tænia tæniæformis* in mice. Thirty-two mice were given 500 onchospheres of *T. tæniæformis* by mouth, twenty-two mice were given 5 mgm. mepacrine for two days before infection and thereafter on alternate days for four weeks. While control mice showed an average of 119·9 living cysts and 9·2 dead cysts, the treated mice had 12·7 living cysts and 23·7 dead cysts. Barrelet (1948) claims good results with mepacrine in human beings, 0·8 gm. being followed by magnesium sulphate, 30 mgm., four hours later. Neghme and Faiguenbaum (1947) and Halawani *et al.* (1948) obtained similar results in dogs and man. *T. cœnurus* and *Dipylidium caninum* were eradicated. Berberian (1946) employed another acridine preparation, 3-chloro-7-methoxy-9-(2′-hydroxy-3′-diethyl-amino) propyl-amino-acridinedihydrochloride, acranil. Twenty-five children with *Hymenolepis nana* infection were treated with varying doses according to age, those from four to eight receiving 0·1 to 0·2 gm., those from eight to ten years 0·3 gm., those from eleven to fourteen 0·4 gm., and those of fourteen years and over 0·5 gm. On the previous evening calomel was administered, and three hours after the drug a dose of sodium sulphate. Examination of the children showed that four weeks later seven of twenty-five children were passing eggs of *H. nana;* however, five months later twelve of the twenty-five were again infected.

Cyanine dyes have been studied by Hales and Welch (1947), the most active being 6-dimethylamino, 2, 5-dimethyl-1-phenyl-3-pyrrole (1-methyl-2-quinoline). A single oral dose of 20 mgm. per kgm. of body weight destroyed all ascaris in eleven out of twelve dogs harbouring *Toxocara canis* and *Toxascaris leonina.* With hookworms in dogs results were very variable, while *Trichuris vulpis* and *Dipylidium caninum* were not affected. Slight reversible lesions were found in the kidneys of dogs after repeated administration, and in a third of the dogs vomiting was seen.

(2) Other Anthelminthics

A number of other compounds, some new, others old, have recently been investigated.

Cestode Infections

Pelletierine is the name of an extract of the bark and roots of the pomegranate *Punica granatum* Linn, which had a reputation as a tænicide in ancient Egypt. Strictly speaking, the name pelletierine should be reserved for the pure optically-active alkaloid isolated by Tanret (1878) from pomegranate root bark. Goodson (1940) has shown that many " pelletierine " salts are apt to be low in L-pelletierine which, there is reason to think, is the active anthelminthic ; *pseudo*pelletierine and the other hydrochlorides of bases liberated by sodium bicarbonate, probably methyl*iso*pelletierine, are not active. Pelletierine tannate, or the French official pelletierine sulphate which is a mixture of total alkaloids from which the weak bases have largely been eliminated, are apparently the most active preparations. The average dose is 0·25 gm., which should be followed two hours later by 1 oz. of castor oil and a soap-and-water enema. In association with tetra-

*iso*butyl tin compounds, pelletierine hydrochloride was found by Guthrie *et al.* (1941) to be highly effective in removing the tapeworm *Raillietina cesticillus* from chickens: the tin compounds alone were ineffective.

Raigan is a Chinese remedy which was well known as a remedy for tapeworm in the first century A.D. It is derived from a mushroom, *Omphalia lapidescens*, and is said by Hiyeda and Terada (1939) to be active against *Tænia solium*, *T. saginata*, *Dipylidium caninum* and *Hymenolepis nana*. In dogs, 20 gm. of the crude product is given three times a day. There is no action on *Ancylostoma* or *Ascaris*.

The effect of **perthiocyanic acid** on the tapeworms of dogs was studied by Enzie (1944a). At a dose rate of 0·1 to 0·2 gm. per lb. of body weight perthiocyanic acid removed all tænia from three dogs and 73 per cent. of *Dipylidium caninum* from eight dogs: at a dose of 0·05 gm. per lb. 42 per cent. of *Tænia* were removed, but no *Dipylidium* were affected. At the highest dose of 0·2 gm. vomiting occurred after a purgative dose of magnesium sulphate, while without a purge there was anorexia, depression and the passage of blood-stained fæces. The total dose in dogs should not exceed 5 gm. No tænicidal action was found in sheep at a dose of 0·1 gm. per lb. of body weight. In dogs no action was found on ascarids, hookworms or whipworms.

Although **arecoline,** derived from the betel or areca nut, was long known in China and was described in the Ming I Beh, written some fourteen hundred years ago (Liu, 1936), it was first recommended for the treatment of hydatids in dogs by Hall and Shillinger (1924). The number of dogs used was comparatively small. In New Zealand, where hydatid disease has been common, it is compulsory to administer the drug to dogs; the effects of arecoline hydrobromide were studied more extensively by Batham (1946). With oral doses of from $\frac{1}{4}$ to $\frac{1}{32}$ gr. (0·016 to 0·002 gm.) per 10 lb. of body weight, 95 per cent. of *Echinococcus* worms were removed. With the larger doses the tendency to convulsions was increased, but the efficiency was not: the recommended dose is therefore $\frac{1}{16}$ gr. (0·004 gm.) per 10 lb. of body weight.

Arecoline hydrobromide was equally active against *Tænia* spp. and *Dipylidium*, but only 50 per cent. of ascarids were removed, while hookworms were unaffected.

The action of arecoline hydrobromide on segments of *Tænia in vitro* showed that in a dilution of 0·001 per cent. the proglottides became relaxed ; the action of arecoline is thus to cause relaxation of worms and subsequent purgation of the host.

Lead arsenate is apparently of some value in dislodging ruminant tapeworms such as *Moniezia expansa* and *M. benedeni*. McCulloch and McCoy (1941) found that 0·5 gm. doses caused the expulsion of tapeworms in lambs and induced gains in weight. Radeleff (1944) gave 0·5 gm. to kids, 0·5 and 1·0 gm. to calves and 2 gm. to cows and obtained similar results, while Hanwood and Guthrie (1940) and Mohler (1940 and 1941) obtained good results in fowls. More recently the drug has been administered to a considerable number of lambs and kids, nearly 4,000 in all (Ward and Scales, 1946a and b ; Habermann and Carlson, 1946 ; Simms, 1947 ; Foster and Habermann, 1948). Few if any comparisons have yet been made of lead arsenate and other tænicides, nor is there available information as to the distribution of the lead or arsenic in the tissues.

Trematode Infections

Few recent advances have been made in the treatment of trematode infections. For *Fasciola hepatica* and *Paragonimus westermani* infections ten to twelve injections of 1 gr. of emetine hydrochloride are recommended (Murashima, 1922 ; Martin, 1927 ; Yokogawa and Ro, 1939 ; Yokogawa *et al.*, 1940). The activity of emetine alone or in combination with prontosil is very irregular, and in many cases it fails to eradicate infection with *Paragonimus*. In dogs infected with *P. kellicotti*, Brown and Hussey (1947) have failed to find any chemotherapeutic action by anthiomaline, neostibosan, methylene violet and *p*-[bis (carboxymethyl mercapto) arsino] benzamide.

For the treatment of liver fluke in cattle Olsen (1944 and 1946) recommends the addition of bentonite to hexachloroethane. A mixture is made of hexachloroethane 500 gm., bentonite 50 gm., white flour a quarter of a teaspoonful, and water 750 ml. Full-grown cattle are given 6½ oz. of the mixture, which has a low toxicity even for debilitated animals; of 209 infected animals, 191, or 91 per cent., were cured. The same mixture in doses of 30 to 60 ml. for a grown animal is of value in sheep infected with *F. hepatica* (Olsen, 1946). Young flukes while still embedded in the liver tissue, before migration to the bile ducts, were not removed. These results have been confirmed by Lapage *et al.* (1947). There is little difference in a bentonite suspension of hexachlorethane (15 mgm. per sheep) and 1 ml. of carbon tetrachloride: an average of 91 per cent. of flukes was destroyed by both anthelminthics (Olsen, 1948).

Nematode Infections

The effect of simple saturated hydrocarbons has been studied by Whitlock (1945) in rats given a standardised infective dose of *Nippostrongylus muris*. Increase in the number of carbon atoms in the side-chain or increased branching of the side-chain decreases the effectiveness of the hydrocarbon. Higher boiling petroleum distillates are ineffective as the constituent hydrocarbons have long, branched chains. Petroleum hexane, synthetic hexane, and *cyclo*hexane are, however, effective. Petroleum hexane mixed with carbon tetrachloride permits a reduced dose of the latter compound, thus decreasing toxicity. Petroleum hexane is as effective as carbon tetrachloride in treating *Hæmonchus contortus*, *Ostertagia*, and *Trichostrongylus* in sheep, but it has a tendency to cause bloat.

While chloroform has long been recognised as having an anthelminthic action, methyl chloroform has also been shown to have an anthelminthic action in dogs. A small series of experiments was carried out by Mohler (1934 and 1936) on dogs and cats when, at a dose of 0·3 ml. per kgm. of body weight, it was found to be 100 per cent. effective against ascarids but a little less active for hookworms. More extensive experiments were carried out by Enzie (1945). Dogs were kept without food for eighteen to twenty-four hours and were then given methyl chloroform in hard gelatin capsules; food was withheld for a further three to four hours. Doses of from 0·1 to 0·5 ml. per lb. of body weight removed 100 per cent. of ascarids, 90 per cent. of hookworms and only 14 per cent. of whipworms. At a dose of 0·1 ml. per lb. of body weight it was 100 per cent. effective on ascarids but at least 0·3 ml. per lb. of body weight is required for a similar action against hookworms. Methyl chloroform has no anthelminthic action in chickens.

For the treatment of round worms and ancylostomes in man a mixture of oil of chenopodium and tetrachloroethylene is still probably the most efficient mixture, although hexylresorcinol is more effective than oil of chenopodium against ascaris. Hexylresorcinol, however, is inactivated by mucus. On the other hand, its action is enhanced by detergents in certain proportions, sodium anacardate, anacardol, the anacid fraction of cashew oil, and a tincture of cashew oil. Sodium anacardate is particularly active and both it and the oil itself have some anthelminthic action (Eichbaum, 1947). There is some evidence that the toxicities of tetrachloroethylene, and oil of chenopodium may be decreased by the addition of ascorbic acid, cystine, glycine, glucuronic acid. The anthelminthic action of ascaridole is also decreased, but the anthelminthic action of tetrachloroethylene is said to be increased by cysteine and methionine (Martin *et al.*, 1941). Guevara (1947) has brought forward evidence to show that the addition of castor oil halves the toxicity of oil of chenopodium.

Indigenous Indian drugs such as butea, embelia, and kamala are reputed to have anthelminthic properties, but only doubtful results have been obtained. Mukerji and Bhaduri (1947), however, have used the seeds of *Butea frondosa*, the dried fruits of *Embelia ribes* and *E. robusta*, and the red glands and hairs of *Malotus philippinensis*, ground to a paste and mixed with sugar and water. For children 8 to 30 gr., for adolescents 40 to 45 gr., and for adults 60 gr. or more were given. In 120 persons with *Ascaris* a single dose of 60 or more grains cured about half ; no curative effects were seen on hookworms or tapeworms. As a single dose of oil of chenopodium is said to cure only thirty-six of sixty-seven persons with round worms, the results are regarded as satisfactory and superior to those of santonin, where only thirty-one of ninety were cured by a single dose (Maplestone and Mukerji, 1931).

For the removal of gape worms, *Syngamus trachea*, from chickens, turkeys and pheasants, barium antimonyl tartrate, if inhaled as a fine dust, appears to be of considerable value. Exposure to the dust is made in a closed box, 24 to 29 gr. per 28 cu. ft. being highly efficient (Weir and Olivier, 1943).

As an alternative to gentian violet in the treatment of oxyuriasis, the carbamic acid ester of *p*-hydroxydiphenylmethane, usually known as *p*-benzylphenyl carbamate (butolan, diphenan, oxylan) is sometimes of value. *In vitro* experiments show that it has a direct vermicidal action ; it also has a low toxicity, although it may cause diarrhœa. It is advisable to precede its administration by giving on each of the three nights before treatment an enema of quassia chips (½ oz. quassia chips to 1 pint of cold water). On the fourth day tablets, which are made up to contain 0·5 gm., are administered ; for infants up to one-and-a-half years quarter of a tablet, for children up to ten years half a tablet, for children over ten years one tablet and for adults two tablets are given three times a day for seven days. At the end of the course castor oil is advisable. In resistant cases it may be necessary to continue treatment for four weeks.

At one period thymol was extensively used for the treatment of hookworms in man, later to be replaced by carbon tetrachloride, but these drugs have now largely been displaced by tetrachloroethylene which, while as efficient as carbon tetrachloride, is considerably less toxic. Since a comparatively simple alteration in chemical structure often produces a change in anthelminthic activity, Enzie (1945) studied the efficiency of eight alkylbenzenes : in addition to thymol, 4-*iso*propyl-*m*-cresol (isothymol) ; 6-methyl-*m*-cresol (4-hydroxy-1, 2-dimethylbenzene) ; 4-tertiarybutyl-*m*-cresol ; 4, 6-di-tertiarybutyl-*m*-cresol ; 4-tertiarybutyl-2-chlorophenol ; 4-tertiaryamylphenol (pentaphen) and 2-amino-*p*-cymene (carvacrylamine) were studied.

The results on ascarids, hookworms and whipworms are shown in the table on p. 164 : 6-methyl-*m*-cresol was not effective and in addition was toxic ; 4-tertiarybutyl-2-chlorophenol was not more efficient than 4-tertiarybutylphenol (butylphen) which had previously been studied by Enzie (1944b).

Creeping eruption, which is now recognised both in Africa and America as being due to penetration of the skin by the larvæ of *Ancylostoma braziliense* and *A. caninum* has in some cases been cured by means of injections of stibophen. Smith (1943) and Rubin (1944) reported favourable results from the intramuscular injection of 2 ml. of a 6·3 per cent. solution of stibophen daily for

ANTHELMINTHIC TESTS WITH ALKYBENZENES IN DOGS (Enzie, 1945)

Compound.	Chemical formula.	Dosage in gm. per lb. of body weight.	Percentage efficiency against		
			Hook-worms.	Asca-rids.	Whip-worms.
6-methyl-*m*-cresol.	$(CH_3)_2C_6H_3OH$	0·1	0	0	0
6-*iso*propyl-*m*-cresol.	$CH_3(C_3H_7)C_6H_3OH$	0·3 to 0·5 0·4 to 0·5	6 —	93 100	13 —
4-*iso*propyl-*m*-cresol.	$CH_3(C_3H_7)C_6H_3OH$	0·2	97	100	69
4-tertiarybutyl-*m*-cresol.	$CH_3[(CH_3)_3C]C_6H_3OH$	0·2 to 0·3	81	99	34
4, 6-di-tertiary-butyl-*m*-cresol	$CH_3[(CH_3)_3C]_2C_6H_2OH$	0·1 0·2	0 0	0 0	0 0
4-tertiarybutyl-2-chlorophenol	$Cl[(CH_3)_3C]C_6H_3OH$	0·1 0·2	67 84	100 100	— 0
4-tertiaryamyl-phenol.	$CH_3CH_2C(CH_3)_2C_6H_4OH$	0·2 to 0·3	94	99	23
2-amino-*p*-cymene.	$(CH_3)_2CH(CH_3)C_6H_3NH_2$	0·075	37	98	0

five days followed, after an interval of a week, by a second course. Usually during the second course all signs of infection have disappeared. Blank (1943), on the other hand, was unable to demonstrate any action from injections of stibophen. Hitch (1947) found oxophenarsine hydrochloride, "mapharsen," more efficacious than antimony preparations. Carbon dioxide snow is, however, probably the most effective remedy available.

It is possible that a deficiency of vitamins in the diet reduces the peristaltic contractions of the intestine and thus increases the emptying time of the bowel. Larsh (1947) has shown that in mice given varying doses of opium before infection there is a decreased rate of emptying of the bowel and an increased development of *Hymenolepsis nana* var. *fraterna* in the gut.

Sodium fluoride has been shown by Allen (1945) to be a highly efficient ascaricide in swine when given for one or two days in the food to the extent of 1 per cent. of the diet. Sodium fluoride in fact is superior to santonin, oil of chenopodium, and phenothiazine for eradicating ascaris from swine (Enzie *et al.*, 1945). Foster *et al.* (1948) find that it eliminates 95 per cent. of *Ascaris* and 93 per cent. of stomach worms, both mature and immature worms ;

it is relatively ineffective against nodular worms and whipworms. Vomiting and diarrhœa are seen in about 10 per cent. of swine. It must not be given in slops or milk. At levels high enough to be effective against chicken roundworms, *Ascaridia galli*, it is toxic to mature birds.

No drug has yet been found with an action against *Trichinella spiralis*. Oliver-González and Hewitt (1947), however, have found that in infected rats an oral dose of 200 mgm. per kgm. weight of 1-diethylcarbamyl-4-methylpiperazine (hetrazan) three times a day reduces the number of worms in the intestine and hence, if given early in the infection for five or ten days, materially reduces the number of larvæ recovered from the muscles.

Hetrazan also reduces the number of ascarids harboured by men and dogs.

Hewitt *et al.* (1948) have employed a number of different dose schedules in dogs, the results being shown in the table. About one half of the dogs vomited after the first or second dose. Dogs given 25·0 mgm. per kgm. of body weight three times daily for from two weeks to two months gained in weight and no lesions were found at necropsy. The drug appears to be rapidly excreted and does not irritate the intestinal mucosa : it may also be given intraperitoneally. Hetrazan appears to be useless for hookworms, whipworms or tapeworms.

THE EFFECT OF 1-DIETHYLCARBAMYL-4-METHYLPIPERAZINE HYDROCHLORIDE ON ASCARIASIS IN DOGS (Hewitt *et al.*, 1948)

Dose (mgm. per kgm.).	Number of dogs used.	Total number of ascarids passed.	Ascarids in intestine at autopsy.	Efficiency of treatment (per cent.).
12·5 × 2 oral . .	6	52	7	88·2
25·0 × 1 ,, . .	7	25	13	65·7
25·0 × 2 ,, . .	7	62	0	100·0
30·0 × 1 ,, . .	6	11	1	91·7
40·0 × 1 ,, . .	7	56	18	75·7
50·0 × 1 ,, . .	10	72	1	98·7
50·0 × 2 ,, . .	4	33	0	100·0
50·0 × 2 intraperitoneal	4	9 (plus)	0	100·0

Anthelminthic Action of Toluene Compounds given to Dogs at the Rate of 0·2 ml. per lb. of Body Weight (Enzie, 1947)

Drug.	Chemical formula.	Parasites.			Efficacy.
			Removed.	Left.	per cent.
Toluene	$CH_3C_6H_5$	Ascarids	25	0	100
		Hookworms	11	0	100
		Whipworms	122	20	85
o-Chlorotoluene	$CH_3C_6H_4Cl$	Ascarids	66	0	100
		Hookworms	38	0	100
		Whipworms	183	17	91
m-Chlorotoluene	,,	Ascarids	23	2	92
		Hookworms	38	2	95
		Whipworms	130	52	71
p-Chlorotoluene	,,	Ascarids	12	10	54
		Hookworms	51	10	83
		Whipworms	166	2	98
o-Bromotoluene	$CH_3C_6H_4Br$	Ascarids	17	0	100
		Hookworms	141	5	96
		Whipworms	374	5	98
m-Bromotoluene	,,	Ascarids	8	2	80
		Hookworms	37	1	97
		Whipworms	95	56	62
p-Bromotoluene	,,	Ascarids	4	0	100
		Hookworms	12	0	100
		Whipworms	31	0	100
o-Iodotoluene	$CH_3C_6H_4I$	Ascarids	—	—	—
		Hookworms	112	1	99
		Whipworms	13	35	27
m-Iodotoluene	,,	Ascarids	1	0	100
		Hookworms	26	67	27
		Whipworms	12	157	7
p-Iodotoluene	,,	Ascarids	8	2	80
		Hookworms	40	49	44
		Whipworms	62	2	96
o-Fluorotoluene	$CH_3C_6H_4F$	Ascarids	1	0	100
		Hookworms	76	0	100
		Whipworms	68	0	100
m-Fluorotoluene	,,	Ascarids	7	0	100
		Hookworms	176	0	100
		Whipworms	65	0	100
p-Fluorotoluene	,,	Ascarids	6	0	100
		Hookworms	192	0	100
		Whipworms	210	0	100
2-6-Dichlorotoluene	$CH_3C_6H_3Cl_2$	Ascarids	—	—	—
		Hookworms	76	0	100
		Whipworms	49	1	98
2-5-Dibromotoluene	$CH_3C_6H_3Br_2$	Ascarids	—	—	—
		Hookworms	46	72	38
		Whipworms	—	—	—

Although the administration of an adequate supply of vitamins in the diet does not have an anthelminthic action, deficiency both of vitamin A and of the B complex lowers the resistance to primary worm infection and also reduces the production of immune bodies (Watt, 1944).

PERCENTAGE OF PIG ASCARIS KILLED BY DIFFERENT LENGTHS OF EXPOSURE TO 1 : 5,000 SALINE SUSPENSION OF DRUG AT 37·5° C. (Williams *et al.*, 1949)

	Time of exposure in minutes.			
	1	2	5	10
2-Ethyl-4-chloro-hexylresorcinol	43	100	100	100
Hexylresorcinol . . .	0	8	56	100

PERCENTAGE OF PIG ASCARIS KILLED BY EXPOSURES TO A 1 : 1,000 SALINE SUSPENSION OF THE DRUG AT 37·5° C. (Williams *et al.*, 1949)

Drug.	Time of exposure in minutes.			
	1	2	5	10
2, 4-Dichloro-6-hexylphenol . .	0	0	0	0
4-Chloro-6-hexyl-*m*-cresol . .	0	0	0	0
2-Ethyl-4-hexyl-6-chloro-*m*-cresol .	0	0	50	100
2, 4-Dihexyl-6-chloro-*m*-cresol .	0	0	0	50
2-Bromo-4-hexyl-6-chloro-*m*-cresol	0	0	0	0
Hexylresorcinol . . .	50	100	100	100
Resorcinol-4-hexylketone . .	0	50	100	100
4, 6-Dihexylresorcinol . . .	50	100	100	100
4-Chloro-6-heptylresorcinol . .	0	50	100	100
2-Hexyl-4, 6-dichlororesorcinol .	0	0	0	0
2-Hexyl-4, 6-dibromoresorcinol .	0	0	0	100
2-Ethyl-4-chloro-6-hexylresorcinol .	100	100	100	100
2-Hexyl-4-chloro-6-acetylresorcinol	0	0	0	0

As examples of comparatively **simple benzene derivatives,** Enzie (1947) has carried out a number of tests with toluene (methyl benzene) and certain halogenated substitution products on ascaris, hookworms and whipworms in dogs. Data on the efficacy of these compounds is shown in the table on p. 166. It will be seen that the introduction of halogens into the ring usually caused a reduction in efficacy. The diminution in activity was more pronounced for hookworms than for ascarids and was considerably greater with the disubstituted products than with the corresponding monohalogenated compounds. In the monohalogenated compounds the *meta*-position was the most favourable site for halogenation, the *para*-position the least favourable.

Halogen-substituted hydroxybenzenes were studied by Hartman and Schelling (1939) for antiseptic properties; some substances had a high germicidal activity. Williams *et al.* (1949) tested these

THE EFFECT ON INFECTIONS WITH *N. muris* OF COMPOUNDS RELATED TO TRICHLOROACETAMIDE. TREATMENT BY DRUG-DIET METHOD FROM DAY OF INOCULATION TO TIME OF AUTOPSY EIGHT DAYS LATER (Brackett and Bliznick, 1949)

Compound.	Dosage in diet (per cent.).	Average number of worms in test : control mice.
Trichloroaceto-*p*-toluide	0·5	1 : 112
	0·3	10 : 112
	0·1	4 : 109
N-(chloroacetyl) trichloroacetamide	0·5	0 : many
Chloral hydrate	0·5	0 : many
	0·1	7 : many
Chloral (stabilised with hydroquinone)	0·5	1 : 41
	0·3	20 : 41
	0·1	30 : 41
Ethyl trichloroacetate	0·3	0 : 99
	0·1	5 : 99
Chloral alcoholate	0·5	0 : 70
	0·3	0 : 99
	0·1	21 : 41
Dichloroacetamide	0·5	many : 198
Chloroacetamide	0·1	many : many
Acetamide	0·5	many : 52
Trifluoracetamide	0·4	many : many
Chloral cyanohydrin	0·1	many : 70
Trichloro-tert-butyl alcohol	0·1	many : many

hydroxybenzenes for anthelminthic properties either by the *in vitro* method of Lamson and Brown (1936) or the kymographic method of Baldwin (1943). The results obtained by exposing porcine ascaris to 1 : 1,000 saline suspensions of the drugs at 37·5° C. for from one to ten minutes are shown in the table on p. 167.

One compound, 2-ethyl-4-chloro-6-hexylresorcinol, has an activity not unlike that of hexylresorcinol.

The oral LD 50 of 2-ethyl-4-chloro-6-hexylresorcinol in rats is very similar to that of hexylresorcinol, namely 0·35 gm. per kgm. : as is the case with other resorcinols, it has a slight escharotic effect on the skin and mucous membranes.

Trichloroacetamide. The anthelminthic activity of N-(chloroacetyl) trichloroacetamide and related compounds against *Nippostrongylus muris* in the mouse has been investigated by Brackett and Bliznick (1949). N-(chloroacetyl) trichloroacetamide given in a dose as small as 200 mgm. per kgm. per day is of considerable value in preventing infection, but its activity against later forms is much less. The activity of this group of compounds is shown in the table on p. 168.

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THE MODE OF ACTION OF ANTHELMINTHIC COMPOUNDS

Despite the wide variety of drugs which show anthelminthic action, remarkably little is known of the true mode of action of drugs on worms. The little that has been found out has been discovered very largely by work on nematodes.

Lamson and Ward (1932) classified anthelminthic action as follows :—

(1) Temporary narcosis or paralysis followed by recovery.

(2) Narcosis or paralysis followed by death.

(3) Injury to the cuticle.

(4) Digestion.

(5) Unexplained death.

In addition there may be a specific toxic action on the genital organs thus suppressing reproduction, or the anthelminthic may interfere with the worm's supply of essential metabolites. The last possibility has as yet hardly been investigated.

Before anthelminthic action can occur, at any rate in the majority of cases, the drug must have penetrated the cuticle. The cuticle of *Ascaris*, for example, is known to display a highly selective permeability (Trim, 1944). Once penetration has occurred the drug must have a deleterious action on the tissues of the helminth.

In nematodes, at any rate, there is a sharp positive correlation between anthelminthic potency and action on the neuromuscular apparatus of the worm (Baldwin, 1943a); the only two important drugs which fail to act on the neuromuscular apparatus are gentian violet and phenothiazine, which may undergo conversion in the host into more active compounds or possibly act on the reproductive organs of the worms.

Although paralysis is the primary action of many anthelminthic drugs, this paralysis may be followed by other phenomena, for example, by contracture with phenolic drugs such as hexylresorcinol, thymol and β-naphthol.

The mode of action of phenothiazine is discussed on p. 127–8 and of miracil D on p. 88.

The action of anthelminthics on the neuromuscular mechanism of *Ascaris* shows very considerable variation. Thus Baldwin (1948), using preparations of the anterior end of *Ascaris*, obtained the results shown in the table on p. 174.

Numerous attempts have been made to correlate anthelminthic action with physical properties and chemical constitution. Much of the earlier work is unsatisfactory because it was carried out with such animals as leeches and earthworms, which belong to an entirely different animal phylum, whereas nematodes display many unique morphological features (Lapage, 1937). It was only when drug action was tested on isolated anterior fragments of *Ascaris* (Rebello and Rico, 1926; Baldwin, 1943a) that consistent results were obtained, for intact *Ascaris* is very resistant to

THE EFFECT OF ANTHELMINTHIC DRUGS IN CAUSING PARALYSIS OF *Ascaris* (Baldwin, 1948)

Compound.	Concentration producing paralysis in 20–30 minutes.	Nature of preparation.
Santonin	1 : 100,000	Solution
Hexylresorcinol	1 : 10,000	,,
p-Benzylphenyl carbamate	1 : 5,000	,,
Thymol	1 : 5,000	,,
β-Naphthol	1 : 5,000	,,
Oil of chenopodium	1 : 5,000	Emulsion
Carbon tetrachloride	1 : 2,000	,,
Carbon tetrachloroethylene	1 : 2,000	,,
Chlorbutol	1 : 1,000	Solution

drug action *in vitro* (von Schroeder, 1885 ; Lamson and Brown 1936). Chance and Mansour (1949) avoided criticisms attendant on the use of portions of worms by employing the whole trematode *Fasciola hepatica.*

By means of a kymographic technique they would divide anthelminthics acting on *Fasciola hepatica* into (1) stimulants ; (2) paralysing compounds, (*a*) rhythmical contractions can be restored by amphetamine 1 in 5,000, (*b*) rhythmical contractions cannot be restored by amphetamine 1 in 5,000 ; (3) lethal drugs.

Of the stimulant drugs some, such as the lactones coumarine and umbelliferone, cause rhythmical movements of large amplitude and low frequency at low concentrations ; amines produce increase in amplitude and frequency after the initial contraction has subsided ; some increase in tone is noted. Chlorinated hydrocarbons with known anthelminthic potency possess stimulant properties on the liver fluke at low concentrations. Hexachlorethane is specially marked in this respect. Whereas in low concentrations the chlorinated hydrocarbons merely stimulate liver flukes, in higher concentrations they are lethal. Some compounds such as phenylurethane and oil of chenopodium paralyse at low concentrations but become lethal as the concentration is raised. Arecoline paralyses *Tænia* as well as liver fluke. The liver fluke appears to be sensitive to all the drugs which affect *Ascaris* and in addition to umbelliferone, pelletierine, extract of male fern, and gentian violet. These differences may be due to fundamental differences in the neuromuscular mechanism of nematodes and trematodes or to differences in the effectiveness of the cuticle as a selective barrier to the penetration of drugs.

Santonin

Although his work is suspect since it was performed on earthworms, Trendelenberg (1916) showed that previously denervated fragments of earthworm muscle contract rhythmically when suspended in Ringer's solution containing santonin. The same effect, which is freely reversible, was evoked by *desmotropo*-santonin, santonin oxime and tetrahydrosantonin, all of which contain the lactone ring present in santonin itself. Santoninic acid, in which the lactone ring is opened, was inert. It was therefore concluded that santonin owed its activity to the presence of the unmodified lactone ring : in support of this theory is the finding that pilocarpine and coumarine similarly lose their characteristic effects if the lactone ring is opened : similarly, santoninic amide, like the acid, is inert. Oswald (1924) pointed out that the physiological activity of many compounds is destroyed by the introduction of a carboxyl group into the molecule and suggested that the inactivity of santoninic acid might be due to the presence of its free carboxyl radical rather than to the absence of the lactone ring. Oshika (1921–22a and b) showed that, at any rate on earthworm muscle, the ethyl esters of santoninic and santonic acids were both active, the corresponding free acids being inactive. Thus the activity of santonin could not be due solely to the lactone ring.

Caius and Mhaskar (1923) administered a number of santonin derivatives to patients infected with *Ascaris* and determined the percentage cured by one test treatment. It will be noted that santoninic acid was active, possibly because under the acid conditions occurring in the stomach the free acid reverts to the lactone. Lactonisation is not however possible either in santonic acid, unless after previous reduction, or in santonous acid, and both of these are active.

THE ANTHELMINTHIC ACTION OF SANTONIN AND ITS DERIVATIVES (5-GRAIN DOSES) (Caius and Mhaskar, 1923)

Drug.	Number of cases treated.	Percentage of worms removed with test treatment.	Number of cases cured by one test treatment.	Percentage cured by one treatment.
Santonin . .	20	87·3	16	80·0
Chromosantonin .	6	27·2	3	50·0
*desmotropo*Santonin .	8	4·5	—	—
Santonone . .	6	—	—	—
Santoninic acid .	26	89·2	19	73·1
Santonic acid . .	19	86·6	16	84·2
Santonous acid .	18	95·0	12	66·6
Photosantonic acid .	11	93·8	9	81·8

Caius and Mhaskar, therefore, came to the conclusion that the active centre is the ketonic group of the unsaturated ring : this is present in santonin, santoninic acid, and santonic acid. Santonous acid is usually figured in the enolic form but it is able to undergo ketonisation for it forms an oxime with hydroxylamine and a hydrazone with phenylhydrazine. *desmotropo*Santonin, which forms neither an oxime nor a hydrazone, does not ketonise and is inactive ; santonone, also inert, likewise possesses no ketonic grouping.

There is also another possibility, that anthelminthic activity is due to the presence of an unsaturated ring. Lautenschläger (1921) and Rosenmund and Schapiro (1934), found that activity of lactones was increased by the introduction of phenyl groups ; Lamson *et al.* (1935b) showed that 4-phenylphenol was much more active than 4-*cyclo*hexylphenol, and Baldwin (1948) observed that among lactones, thiazoles and pyridines the introduction of a phenyl radical leads to increased anthelminthic action. Tetrahydrosantonin was found by Baldwin to be as active as santonin itself.

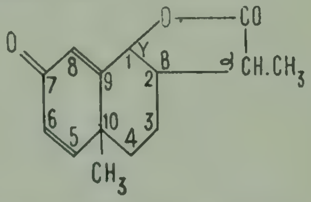
Baldwin (1948) believes that the angular methyl group present in santonin and all its active derivatives may be involved, since such a group is of importance in the sex hormones where the masculinising hormones (androsterone, testosterone) possess two angular methyl radicals and the œstrogenic hormones (œstrone, œstradiol) possess but one: progesterone, which suppresses some of the characteristic features of feminine sexuality, while emphasising others, resembles the androgens in containing two such groups.

Baldwin (1948) points out that in all the active compounds with the exception of santoninic acid, which probably owes its activity to the ease with which it reverts to santonin in aqueous solution, there are three structural features in common. These are :—

(*a*) An intact γ-lactone ring;
(*b*) A double bond at position 7;
(c) An angular methyl group at position 10.

The numbering refers to the following structure :—

One or more of these characters is absent from inert derivatives. The inactivity of santonic acid is probably not due simply to the fact that it contains a free carboxyl radical, for its ethyl ester also is inert; probably, therefore, the inactivity of the acids cannot be due to their free carboxyl groups.



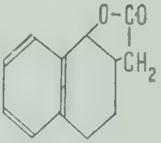
SANTONIN AND ITS DERIVATIVES: ACTIVITY ON THE NEUROMUSCULAR APPARATUS OF *Ascaris* (Baldwin, 1948)

Active	Inactive
Santonin.	D-*desmotropo*Santonin.
β-Santonin.	L-*desmotropo*Santonin.
Santoninic acid.	L-*desmotropo*-β-Santonin.
Tetrahydrosantonin.	D-Santonous acid.
Santonin oxine.	L-Santonous acid.
	Santonic acid.
	Ethyl santonate.
	Allantolactone.
	ϕ-Santonin.

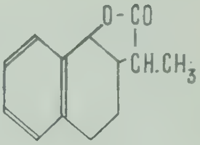
Lactones

Since Trendelenberg (1916) first emphasised the possible importance of the lactone ring in the anthelminthic properties of santonin, many new lactones have been prepared and tested by Lautenschläger (1921), von Oettingen (1929), Gluschke (1932), Rosenmund and Schapiro (1934) and Baldwin (1943a, 1948).

Lautenschläger (1921) tested γ-butyrolactone, γ-valerolactone, paraconic acid lactone and a number of sugar lactones and betaines. The introduction of phenyl radicals increased activity: phenyl butyrolactone and phenyl paraconic acid lactone were about half as active as santonin on earthworm preparations, and phthalide ($\alpha:\beta$-benzbutyrolactone) was as active as santonin itself. von Oettingen (1929) showed that butyrolactone, valerolactone, valerolactone carboxylic acid, *iso*caprolactone, α- and β-angelica lactones and the dilactone of acetone di-acetic acid depressed isolated earthworm muscle, but the introduction of methyl or carboxyl groups into the lactone ring increased activity as did the introduction of a double bond. At concentrations of 0·04 M β-angelica lactone, valerolactone carboxylic acid and the dilactone equalled santonin in activity, though in more dilute solutions the action was not so marked. von Oettingen and Garcia (1929) found that β-angelica lactone removed all the round-worms from seven out of ten infested cats. Still using earthworms, Gluschke (1932) claimed that certain lactones such as syntonins *a* and *b* (I and II) derived from α-tetralone equalled or surpassed santonin in activity :—



(I)

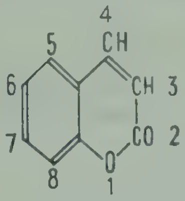


(II)

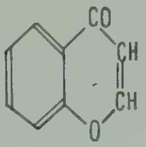
Rosenmund and Schapiro (1934) again prepared a series of substituted γ-butyrolactones, which were tested on intact *Ascaris* and on leech muscle. The *o*-cresol ether and anisole derivatives of γ-butyrolactone were said to be three to four times more active than santonin. The claim was also made that a close parallel exists between the response of leech muscle and of intact round-worms. As Baldwin (1943b, 1948) has pointed out, the validity of Rosenmund and Schapiro's observations must remain uncertain in view of the finding that santonin itself has little or no apparent action on intact *Ascaris* (von Schroeder, 1885; Lamson *et al.*, 1935a, b, c, 1936a, b, c).

The largest series of lactone derivatives has been studied by Baldwin (1943b, 1948), using a far more sensitive *Ascaris* preparation than had been employed by his predecessors. Of α-angelica lactone, its anisal derivative, the dilactone of acetone diacetic acid and copper glycine, only α-angelica lactone showed any activity in a dilution of 1 in 1,000. Neither D- nor L-*desmotropo*santonin nor L-*desmotropo*-β-santonin was active, and the same was true of allantolactone, a substance even more closely allied to santonin than are the syntonins. This lack of activity does not agree with the suggestions put forward by von Oettingen (1929) and Gluschke (1932).

In a series of derivatives of γ-butyrolactone the activity of γ-phenylbutyrolactone is very close to that of santonin in high concentrations, but phenylbutyrolactone gives only dubious or slight activity at 1 in 10,000 whereas santonin is still active in a dilution of 1 in 100,000. Alkyloxylation removed the activity. Baldwin's results are thus at variance with those obtained by Rosenmund and Schapiro (1934).



(III)



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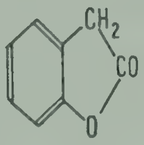

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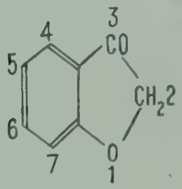
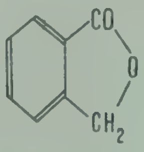


(VI)



(VII)

Coumarine (III) was active, but umbelliferone (7-hydroxycoumarine), 3-hydroxycoumarine, and 7-ethoxycoumarine were inert. Chromone (IV), 2-coumaranone (V), and 3-coumarone (VI) had some activity, but neither they nor derivatives related to phthalide (VII) were as active as santonin or even as phenylbutyrolactone. These findings suggest that higher potencies are associated with separated rather than with fused rings.

All four parent compounds (III—VI) may, as Baldwin (1948) points out, be regarded as cyclised derivatives of *o*-phenols and all possess some activity. The most active member of the group, 3-coumaranone (VIII), can be regarded as a cyclised form of 2-hydroxyacetophenone with the activity of which its own is comparable ; 6-hydroxy-3-coumaranone, which may be compared with 2 : 4-dihydroxyacetophenone, is inert. Hydroxylation of coumarine, at position 7, similarly leads to loss of activity, but replacement of (OH) by an ethoxy radical leads to some degree of activity.

Three phenylated ketolactones derived from butyrolactone were found to be inert, as was clavatin which, according to Raistrick (1943), contains a lactone ring.

It has been suggested by Rosenmund (1935) that the electrical characters of the phenol lactones are of great importance in relation to anthelminthic action. By studying the dipole moments of these compounds and measurement of these moments the physicochemical properties can be predicted.

Thus the appearance of anthelminthic activity among lactones is very sporadic and is quite possibly fortuitous. The outstanding feature is the greater activity of compounds with separated as opposed to fused rings.

Aliphatic-aromatic Ketones

By partial dissections of santonin it is possible to obtain, on paper, benzylidene acetone. This compound and a group of related ketones were found by Baldwin (1948) to possess appreciable anthelminthic activity. Activity is mainly linked with the ketonic group, the presence of one or more unsaturated linkages in the side-chain increasing the activity to some degree. Replacement of the phenyl by a furfuryl radical reduces activity considerably.

Increase in activity was obtained by introducing alkyloxy radicals into position 4 of benzylidene acetone but not of acetophenone. This suggests that the unsaturated side-chain of benzylidene acetone carries greater anthelminthic potentialities than the saturated side-chain of acetophenone. Among homologous alkyloxy derivatives highest activity is found in the 4-ethoxy compound, a fall in potency taking place when the length of this radical is further increased. Maximal activity occurs when the alkyloxy group is in position 4, minimal activity when the alkyloxy group is in position 2. The effect of pairs of alkyloxy radicals is not additive in the acetophenone series and is usually antagonistic in the benzylidene series. The introduction of allyl groups serves only to diminish activity.

Diones, regarded as derived from cinnamylidene acetone, are inert.

Baldwin (1948) pointed out that when, by the introduction of one potentiating group, a relatively high order of potency has been developed, addition of a second potentiating radical is liable to diminish rather than increase it.

Just as the anthelminthic potentialities of resorcinol are augmented by the addition of longer alkyl radicals into the side-chain (Lamson, Brown and Ward, 1935), so Baldwin has found considerable anthelminthic activity in resorcinyl ethyl ketone, whereas resorcinyl methyl ketone is inert. Further increase in intensity occurs as the alkyl chain is lengthened, reaching a maximum in the valeryl ketone ; further lengthening of the chain is attended by diminished activity. In a group of substances derived from benzophenone and containing two aromatic rings, the effects of hydroxylation at positions 2 and 4 are precisely opposite to those observed in the acetophenone series.

The relationship between chemical structure and anthelminthic activity in ketones may be summarised as follows (Baldwin, 1948) :—

(1) The ketonic group of aliphatic-aromatic ketones carries potentialities for anthelminthic activity which are nearly equal to those of thymol and β-naphthol.

(2) The latent potency can be evoked by substitution of alkyloxy or phenolic radicals, in the benzene ring or by halogenation.

(3) The influence of these potentiating radicals varies from one group of ketones to another and with the position of the substitution.

(4) The effects of these potentiating radicals are not additive and may be antagonistic in some compounds.

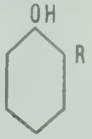
Phenols and Phenolic Derivatives

Much attention has been paid to the anthelminthic action of phenol and phenolic derivatives in relation to chemical structure in view of the fact that thymol (3-methyl-6-*iso*-propylphenol), β-naphthol and hexylresorcinol (2 : 4-dihydroxy-*n*-hexylbenzene) are all active. In an attempt to obtain compounds equally toxic to *Ascaris* but less irritant locally, Lamson and his colleagues (1935a, b, c ; 1936a, b, c) examined the ascaricidal action *in vitro* of series of alkylresorcinols, alkylcresols, alkylphenols, polyalkylphenols, phenols with other than normal alkyl side-chains, alkyl-polycyclicphenols, halogenated phenols, phenolic ketones, phenolic ethers and esters and a number of organic acids.

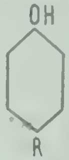
The loss of activity on the part of heptylresorcinol as compared with hexylresorcinol may be due in part, according to Lamson *et al.* (1932), to the fact that it is hardly absorbed at all by the cells of the intestine and possibly hardly at all by *Ascaris*.

Among a group of miscellaneous polyhydroxybenzenes, 4-*n*-hexylcatechol was the only one with any marked ascaricidal action *in vitro*. In the *ortho*- and *para*-*n*-alkylphenol series ascaricidal properties increased with the introduction of normal alkyl radicals into both the *ortho*- and *para*-positions of phenol up to and including *n*-amylphenol ; in the higher members of the series ascaricidal action rapidly diminished. Coulthard *et al.* (1930), it is of interest to note, similarly found an increase in bactericidal action and a decrease in toxicity with increase in length of the side-chains in the *n*-alkylphenol series.

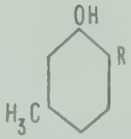
Whereas the *in vitro* ascaricidal properties of the *ortho*- and *para*-alkylphenols are very nearly the same, the *para*-compounds are consistently slightly the more active, but the *ortho*- series are less irritating to mucous membranes.



ortho-n-alkylphenols



para-n-alkylphenols



6-n alkyl m cresols

In the 6-*n*-alkyl-*m*-cresols also Lamson and Brown (1935) showed that ascaricidal action increases with length of the alkyl radical from *m*-cresol up to butyl-*m*-cresol, after which activity falls off. The toxicity of the series decreases with increase of the alkyl radical, as does the local irritant action, 6-*n*-hexyl-*m*-cresol being the lowest member of the series which does not cause whitening of the oral mucosa.

Among polyalkylphenols, Lamson, Brown and Ward (1935) found some which showed considerable activity *in vitro*, but no single substance combined in itself high ascaricidal activity against pig *Ascaris*, low toxicity, and inapparent local irritant action to such a degree as to indicate that it would be a more practical anthelminthic than hexylresorcinol.

The introduction of alkyl radicals into certain polycyclic phenols leads to no increase in ascaricidal action, but the introduction of halogens into certain alkylphenols is more promising and ascaricidal action *in vitro* is particularly shown by *p*-chlorocarvacrol, 4-chloro-2-hexylphenol and 4-chloro-2-heptylphenol. Kochmann (1931) found that *in vitro Ascaris* is paralysed by concentrations of 1 in 150,000.

Certain other alkylpolyhydroxyhalogenobenzenes such as 1-octyl-5-chlororesorcinol, or 5-octyl-4-chloropyrocatechol (British Patent 415715 : 1934) may also possess some anthelminthic action.

Ascaricidal action was not shown by more than 100 phenolic ketones, ethers and esters, though some thirty organic acids and twenty esters of simple aromatic and aliphatic acids had a very slight ascaricidal action. Two monoethers of dihydric phenols, namely quinol monoamyl ether and hexylresorcinol monoethyl ether, were highly ascaricidal *in vitro* but unfortunately were too toxic for use *in vivo*.

These observations have been continued by Baldwin (1948), but unfortunately *in vitro* activity on *Ascaris* was not correlated with toxicity. A number of phenolic acetates, chloroacetates, methylsulphonates, benzenesulphonates, *p*-toluenesulphonates, cinnates and carbamates proved either inert or insoluble ; of the carbamates, however, 2-naphthyl carbamate was active in a dilution of 1 in 5,000, 2-hydroxydiphenyl carbamate in a dilution of 1 in 20,000.

As the carbamates showed activity of the same order as the parent phenols and are of lesser toxicity, they may be of practical use.

Among lactones previously examined greater activity has been found in those containing independent than in those containing fused ring systems. To determine whether the same rule applied among the phenols, a number of phenylated phenols were studied by Baldwin (1948). One member of this series, 4-benzylphenyl carbamate, is already in use under proprietary names and has a certain reputation in the treatment of oxyurids. The results are shown in the table, where the most useful derivatives are the carbamates.

THE ACTION OF PHENYLATED PHENOLS ON *Ascaris in vitro*
(Baldwin, 1948)

Compound.	Activity.	Dilution.
$2\text{-}HO\,.\,C_6H_4C_6H_5$	+ +	1 : 5,000
	+	1 : 10,000
$2\text{-}C_2H_5O\,.\,C_6H_4C_6H_5$	—	1 : 1,000
$2\text{-}CH_3CO\,.\,O\,.\,C_6H_4C_6H_5$	+ +	1 : 10,000
	(+)	1 : 20,000
$2\text{-}CH_3SO_2O\,.\,C_6H_4C_6H_5$	—	ca. 1 : 2,000
$2\text{-}C_6H_5SO_2O\,.\,C_6H_4C_6H_5$	insoluble	
$2\text{-}(4'\text{-}CH_3\,.\,C_6H_4SO_2)O\,.\,C_6H_4C_6H_5$	insoluble	
$2\text{-}C_6H_5CH : CH\,.\,CO\,.\,O\,.\,C_6H_4C_6H_5$	insoluble	
$4\text{-}HO\,.\,C_6H_4C_6H_5$	insoluble	
$4\text{-}C_2H_5O\,.\,C_6H_4C_6H_5$	—	1 : 2,000
$4\text{-}CH_3CO\,.\,O\,.\,C_6H_4C_6H_5$	insoluble	
$4\text{-}H_2N\,.\,CO\,.\,O\,.\,C_6H_4C_6H_5$	—	1 : 5,000
$2\text{-}HO\,.\,C_6H_4CH_2C_6H_5$	+	1 : 5,000
$2\text{-}C_2H_5O\,.\,C_6H_4CH_2C_6H_5$	—	1 : 2,000
$4\text{-}HO\,.\,C_6H_4CH_2C_6H_5$	+ + +	1 : 2,000
	+ + +	1 : 5,000
$4\text{-}C_2H_5O\,.\,C_6H_4CH_2C_6H_5$	—	1 : 2,000
$4\text{-}H_2N\,.\,CO\,.\,O\,.\,C_6H_4CH_2C_6H_5$	+ +	1 : 5,000
$4\,.\,CH_3SO_2O\,.\,C_6H_4CH_2C_6H_5$	—	1 : 1,000

+++ producing paralysis in 10 minutes.
++ ,, ,, ,, 10 to 20 minutes.
+ ,, ,, ,, 20 to 30 minutes.
(+) ,, ,, ,, > 30 minutes.
+ Very weak or doubtful.
— Inactive.

Certain relationships appeared especially with reference to the position of the phenolic (OH) group.

2-Hydroxydiphenyl was active at 1 in 10,000 and the 4-compounds insoluble; these results do not confirm Lamson *et al.* (1935c), who found the 4-compound strongly, and the 2-derivative only feebly, active. In the diphenylmethane series these effects were reversed (Lamson *et al.*, 1935a; Baldwin, 1948). In contrast to the findings among the ketones, among phenylphenols and benzylphenols replacement of (OH) by an ethoxy radical, whether in the 2- or 4-position, abolished activity. Compared with 1-naphthol (+ at 1 in 5,000) with its fused rings, 2-hydroxydiphenyl, with independent rings, gave a higher order of activity (+ + at 1 in 5,000), thus falling into line with the finding among lactones. Among the phenylphenols and benzylphenols the active hydroxy derivatives showed higher potencies than that of 1- and 2-naphthols. 2-Hydroxydiphenyl carbamate, the most potent compound, was considerably more active than the parent phenol: 4-benzylphenyl carbamate, on the other hand, is less active than the phenol from which it is derived.

The following conclusions may therefore be drawn from these observations:—

(1) With increase of the alkyl side-chain there is invariably an increase in ascaricidal activity to a maximum followed by a rapid loss of activity. In the series 4-*n*-alkylresorcinols, resorcinol and propylresorcinol are practically inactive against pig *Ascaris in vitro*, butylresorcinol possesses slight activity, and amyl-, hexyl- and heptylresorcinol are all markedly ascaricidal. With octylresorcinol the activity decreases and is absent in duodecylresorcinol and higher members of the series.

(2) The position of the (OH) radical has different effects in different chemical groups.

(3) Phenols containing independent ring systems are more active than those in which the rings are condensed.

(4) Substances with good ascaricidal activity may have a wide range of solubility in water, varying from 1 : 1,000 to 1 : 35,000.

(5) Melting point is probably of greater importance than solubility for, as Harwood (1934) has pointed out, all those com-

pounds which show activity have relatively low melting points, usually below 80° C.

(6) At any rate, with the resorcinols a certain degree of local irritant action is inseparable from anthelminthic action. The lower members of the series have, as a rule, a more intense local irritant action than the higher members and, according to Brown and Lamson (1935), the lower members of the series are more toxic to rats than the higher, although Anderson *et al.* (1931) found that in guinea-pigs hexyl-, heptyl- and octylresorcinol increased in toxicity in that order when given by mouth.

Thiazoles

A series of thiazoles, beginning with 2-aminothiazole, was examined *in vitro* by Baldwin (1948); this compound was inactive, but appreciable activity appeared with the introduction of a phenyl radical to form 2-amino-4-phenylthiazole. Hydroxylation of the benzene ring rendered the compounds inactive. Sulphathiazole was quite inactive. Benzthiazole was active (+ + 1 in 2,000), but 2-phenylbenzthiazole inactive.

Pyridines

The *in vitro* activity of pyridine and a number of pyridine derivatives was investigated by Baldwin (1948): the results are shown in the table opposite.

Pyridine showed an activity of the same order as that of thymol and β-naphthol. Of substituted products only 2-aminopyridine showed greater activity. With the introduction of a second ring to form 4-benzylpyridine, activity increased but sulphapyridine was inert.

A series of dipyridyls showed high activity, 2-2′-dipyridyl having an activity comparable with that of santonin. The results are shown in the table on page 188.

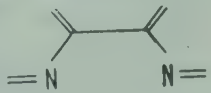
This high potency is apparently associated with the 2-2′-linkage, for when this is shifted to the 2-3′-position there is a considerable fall in potency: 2-2′-2″-tripyridyl is rather less active than the dipyridyl at the same concentrations; the corresponding tetrapyridyl is insoluble.

THE ACTION OF PYRIDINE AND PYRIDINE DERIVATIVES ON *Ascaris in vitro* (Baldwin, 1948)

Compound.	Activity.	Dilution.
Pyridine	+ + +	1 in 2,000
	+	1 in 5,000
2-Methylpyridine	+ + +	1 in 2,000
	(+)	1 in 5,000
2-Chloropyridine	+ +	1 in 1,000
	±	1 in 2,000
1-Methyl-2-pyridine	—	1 in 1,000
2-Aminopyridine	+ + +	1 in 2,000
	+ + +	1 in 5,000
	—	1 in 10,000
4-Benzylpyridine	+ + +	1 in 5,000
	+	1 in 10,000
2-(4′-Aminobenzenesulphonamido)-pyridine (Sulphapyridine) . . .	—	1 in 1,000
Arecoline (1-Methyl-Δ^3-tetrahydro-pyridine-3-carboxylic methyl ester) .	+ +	1 in 1,000
Nikethamide (Pyridine-3-carboxylic diethylamide)	—	1 in 1,000

+ + + producing paralysis in 10 minutes.
+ + ,, ,, ,, 10 to 20 minutes.
+ ,, ,, ,, 20 to 30 minutes.
(+) ,, ,, ,, in 30 minutes.
± Very weak or doubtful.
— Inactive.

Baldwin (1948) next studied a number of compounds containing pairs of nitrogen atoms linked to adjacent carbon atoms, but activity was found only in the phenanthrolines. 4 : 5-Phenanthroline was able to produce paralysis of *Ascaris* muscle in ten to twenty minutes in a dilution of 1 in 100,000, being thus equal to 2-2′-dipyridyl. It is therefore possible that a high order of anthelminthic potency is associated with the bond system, which is common to the two most active compounds. Both 2-2′-dipyridyl and 4 : 5-phenanthroline are probably too soluble for use as anthelminthics against intestinal nematodes, but if not too toxic they may form a new



starting point in the search for anthelminthics for helminthic infestations of the blood and lymphatic systems.

THE ACTION OF DIPYRIDYL DERIVATIVES ON *Ascaris in vitro*
(Baldwin, 1948)

Compound.	Activity.*	Dilution.
2-2′-Dipyridyl	+ + +	1 in 50,000
	+ +	1 in 100,000
2-3′-Dipyridyl	+	1 in 1,000
3-4′-Dipyridyl	±	1 in 1,000
4-4′-Dipyridyl	±	1 in 1,000
2-Methyl-4-4′-dipyridyl	—	1 in 1,000
4-Pyridylpyridinium chloride	—	1 in 1,000
2-2′-2″-Tripyridyl	+ +	1 in 50,000
	±	1 in 100,000
2-2′-2″-2′″-Tetrapyridyl	insoluble	

* Symbols as in two previous tables.

Halogen Derivatives of Carbon

Since the original discovery that chloroform had anthelminthic properties, much consideration has been given to the halogen derivatives of carbon; carbon tetrachloride and tetrachloroethylene (C_2Cl_4) are now two of the most widely used anthelminthics and were introduced largely on the grounds that the activity of chloroform depended on its halogen content. The first extensive experiments on the relationship between chemical constitution and physical properties on the one hand and anthelminthic action on the other were by Wright and Schaffer (1929, 1931, 1932). The correlation was not always very close. Increase in the length of the hydrocarbon chain in normal monochloro-compounds led neither to a progressive rise nor fall in efficiency, for *n*-butyl chloride was the most efficient member, but *n*-hexyl chloride was more effective than *n*-amyl chloride. Increasing the length of the hydrocarbon chain in secondary monochloro-compounds did, however, increase anthelminthic efficiency, for 2-chloropentane was nearly five times as efficient in removing hookworms from the dog as 2-chlorobutane; the increased efficiency is here associated with decreased water solubility.

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CHAPTER IV

THE CHEMOTHERAPY OF AMŒBIASIS

WHILE continued progress has been made in the specific treatment of most protozoal infections, in amœbiasis there has been for some years little important progress. During the first world war it was fully established that injections of emetine hydrochloride alone fail to cure a considerable percentage of cases of amœbic dysentery. These conclusions were strongly reinforced by the results of treatment during the 1933 outbreak of amœbiasis in Chicago, which in all probability, however, was mainly a bacillary outbreak in a group of people with a high carrier rate of *Entamœba histolytica* such as was, and still is, found in Chicago and other American cities (Wenyon, 1934).

Amœbicidal action may occur with a number of compounds, but only the alkaloids of ipecacuanha, certain oxyquinolines, and to a lesser extent quinquevalent arsenicals, have found a definite place in the chemotherapy of amœbiasis.

The ideal amœbicidal compound, which has yet to be found, should rapidly destroy parasitic amœbæ both in the intestine and in the tissues: it should be active when given by mouth and should be readily absorbed from the lumen of the bowel. Finally, it should be non-toxic even after prolonged administration.

In the early part of the second world war it was obvious that many of the rules for the specific treatment of amœbiasis, rules painfully learnt during the years 1914–18, had been forgotten. Fresh amœbicidal compounds are urgently needed, but even with the drugs now at hand it is possible to cure a very high percentage of cases of amœbiasis provided only that the available drugs are properly applied.

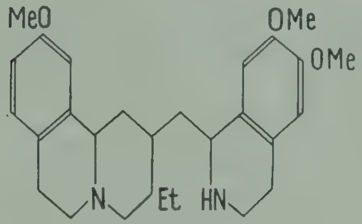
THE ALKALOIDS OF IPECACUANHA

The medicinal qualities of ipecacuanha root were apparently known before the Spanish conquest to the natives of Brazil.

whence it was introduced to Europe by Guillaume le Pois (Guilielmus Piso, 1658). The virtues of ipecacuanha were, however, mentioned as early as 1625 by Samuel Purchas in " Purchas his Pilgrimes." A few years later it was being used in India by the Portuguese, and towards the end of the century Helvetius successfully employed ipecacuanha in the treatment of the Dauphin, son of Louis XIV. Thomas Dover (1733) made ipecacuanha one of the ingredients of his famous powder, and Cockburn (1736) recommended it in some forms of looseness of the bowels, its value being due, it was thought, to its emetic action. It was not till the nineteenth century that powdered ipecacuanha became widely used in the treatment of dysentery. Docker (1858), for instance, reported that its use among troops in Mauritius over a period of some years, in doses of 60 to 90 gr. (4 to 6 gm.) by mouth two or three times a day, produced a considerable reduction in the mortality from dysentery. Parkes (1846) had been employing it in India at an even earlier date.

The roots of *Cephaëlis ipecacuanha* (Brot.) A. Rich constitute the ipecacuanha of commerce, and probably also that obtained from Johore, in Malaya. Not less than two-thirds of the total alkaloids should be non-phenolic. Brazilian ipecacuanha has 72 per cent. of its alkaloids in the form of emetine, Indian ipecacuanha 70 per cent. (Chopra and Mukherjee, 1932). A second commercial variety known as Carthagena ipecacuanha is believed to be derived from *C. acuminata*, which flourishes in Colombia. The optimum œcological conditions for the growth of *C. ipecacuanha* are described by Veloso (1947). Rain forest with a high degree of shade and well-drained slightly acid soil is required. The alkaloid emetine, though first isolated by Pelletier and Magendie (1817), was obtained in a pure state only in 1894 by Paul and Cownley, who separated from commercial emetine first the phenolic base cephaeline and later a third alkaloid, psychotrine. To these Pyman (1917) added emetamine and *o*-methylpsychotrine. There is some evidence that emetine as obtained commercially may contain varying amounts of cephaeline. The structural formulæ of all the alkaloids was studied by Brindley and Pyman (1927), who showed that they were complex *iso-*

quinolines. Robinson (1948) suggests that the structural formula of emetine is



Emetine

The question is further discussed by Battersby *et al.* (1949).

The Pharmacology of Emetine

Emetine, if directly applied to the cornea, to mucous surfaces, or to the tongue, has, according to Chopra *et al.* (1927–28), an irritant action. In the mouth it causes an increased flow of saliva but the amount of amylase is decreased. Peptic digestion, on the other hand, is stimulated by dilutions higher than 1 in 2,000. When given intravenously, the tone and movement of the alimentary tract are stimulated by direct action of the alkaloid on the musculature of the tract, the nervous mechanism being unaffected ; in large doses the intestinal musculature is relaxed and depressed (Epstein, 1932). Both the volume and automatic movements of the spleen are augmented while there is increased vascularity of the gut (Ghosh and Adhya, 1943). This pooling of blood in the splanchnic region, the area chiefly parasitised by *Entamœba histolytica*, possibly helps the amœbicidal action of emetine. Chopra *et al.* (1935) found that the intravenous injection of therapeutic doses of emetine hydrochloride (1 mgm. per kgm.) into Belgian hares caused a reduction in the iodine content of the thyroid and a decrease in the adrenaline content of the adrenals. It is doubtful whether this decrease in adrenalin can explain the fall in blood pressure recorded by Tournade *et al.* (1935). Ghosh and Adhya (1943) observed in man that emetine hydrochloride depressed the auricle more than the ventricle, the action being on the cardiac muscle rather than on the nervous mechanism. The fall in blood pressure may be due in part to depression of the medullary centre, in part to the depressant action on the heart muscle. Both emetine and cephaeline produce marked dilatation of the coronary vessels (Epstein, 1932). Emetine does not appear to stimulate directly the vomiting centre in the medulla (Ghosh and Adhya, 1943), but this is contrary to the findings of Eggleston and Hatcher (1915), who suggested that the emetic effect of emetine is due to both reflex and central stimulation. Cephaeline is said to have a more pronounced emetic action than emetine. Emetine in therapeutic doses does not cause contraction of the uterine muscle and is not therefore likely to cause abortion. Brueckmann and Wertheimer (1945) found that in white rats injections of 2 mgm. of emetine hydrochloride per 100 gm. of body weight caused a fall in liver glycogen. There is no evidence that repeated small doses of emetine impair the liver function; in fact, in patients with amœbic dysentery, a therapeutic course of emetine improves liver function, as demonstrated by Quick's hippuric acid test (Heilig and Visveswar, 1944), probably because cure of the dysenteric lesions prevents further absorption of bacterial toxins. It would seem that emetine is detoxicated comparatively slowly in the body. Unfortunately tests for emetine are hardly sensitive enough to follow the drug adequately in the body. Parmer (1948), however, adapted the general method of Brodie *et al.* (1947) for the estimation of emetine in the livers and intestinal wall of rabbits given intramuscular injections of 6 mgm. per kgm. The concentrations of emetine were as follows :—

Time after injection.	Emetine concentration (mgm. per kgm. of body weight).	
	Liver.	Intestinal wall.
1 hour	24·9	5·5
12 hours	41·6	1·7
3 days	19·2	1·7
4 ,,	15·4	1·2
6 ,,	10·3	0
13 ,,	2·7	0
20 ,,	2·1	0
28 ,,	1·0	0

The high concentration of emetine in the liver and the low concentration in the intestinal wall reveal a striking difference. Amœbicidal activity cannot be demonstrated in the blood of patients after varying doses of emetine given subcutaneously or even in bowel washouts from patients receiving emetine bismuth iodide. Thus it is at present impossible to explain why emetine given parenterally fails to cure intestinal amœbiasis in the kitten.

Toxicity

Emetine is a general cytoplasmic poison and its effects are cumulative, a fact originally demonstrated by Dale (1915) in cats, and since found to apply to other animals and to man. In guinea-pigs, emetine may have cumulative action when given in doses as small as 1/20 M.L.D. (Rosen, Martin and David, 1935). Both in animals and man there is, however, a considerable range in toxicity as a result of individual susceptibility.

The Toxicty of Emetine for Animals

Species.	Mode of administration.	Average lethal dose in mgm. per kgm.	Authority.
Monkey	Subcutaneous	14	Rogers (1914).
Cat	Intravenous	6 to 16	Levy and Rowntree (1916).
,,	,,	6 to 25	Ghosh and Adhya (1943).
,,	Divided subcutaneous (2 to 5 mgm./kgm. daily)	4 to 15	Levy and Rowntree (1916).
,,	Oral	15 to 20	Anderson and Leake (1930).
Rat	Subcutaneous	15 to 20	Lake (1918).
Rabbit	,,	10	Walters and Koch (1917).
,,	,,	14	Rogers (1914).
,,	,,	20 to 25	Lake (1918).
,,	Oral	15 to 20	Anderson and Leake (1930).
,,	Divided intramuscular (1 mgm./kgm. daily)	10 to 12	Chopra *et al.* (1924).
,,	Divided subcutaneous (3 to 4 mgm./kgm. daily)	21 to 44	Levy and Rowntree (1916).
,,	Divided subcutaneous (1 to 5 mgm./kgm. daily)	21 to 25	Walters and Koch (1917).

The toxicity of emetine for animals is shown in the table. As Anderson and Leake (1930) and Leake (1932) point out, the "toxic range" of emetine by intramuscular injection for man and all the larger mammals is relatively constant in the neighbourhood of 10 to 25 mgm. per kgm. of body weight, irrespective of whether the drug is given as a single injection or in divided doses daily. In cats and dogs Boyd and Scherf (1941) found that 37 mgm. per kgm. of body weight, given intravenously, was the largest dose from which animals regularly recovered ; larger amounts caused death from cardiac failure in a few minutes. Guglielmetti (1918) and Levy and Rowntree (1916) regard 4 to 18 mgm. per kgm. as the minimal lethal dose.

Ghosh and Adhya (1943), in cats, found that intravenously a smaller dose was fatal if given in a stronger concentration : 6 mgm. per kgm. of body weight was fatal in a solution containing 2 mgm. per ml. ; 25 mgm. per kgm. killed when the solution contained 0·5 mgm. per ml. Since in man toxic results have been noted with doses of 25 mgm. per kgm. of body weight, it is probably inadvisable to give human beings single doses of more than 1 mgm. per kgm. of body weight or a total of 10 mgm. per kgm. Vedder (1914), for instance, recommended that the average total adult dose should not exceed 0·65 gm.

During the war of 1914–18 large doses of 2 to 3 gr. (120 to 180 mgm.) of emetine hydrochloride were given somewhat indiscriminately to all patients with dysenteric symptoms, although many of these patients were suffering from acute bacillary dysentery. As a result there were many severe reactions and many deaths due to a combination of emetine poisoning and the toxins of bacillary dysentery.

Symptoms of emetine poisoning may include anorexia, nausea, vomiting, abdominal pain and diarrhœa : they are most frequently seen after from four to six injections. Severe hæmorrhages are rare as are more alarming symptoms such as albuminuria, generalised œdema, petechial hæmorrhages, purpuric or urticarial skin rashes with pruriginous spots, hæmoptysis and signs of pulmonary or cerebral œdema (Chopra, 1934). Wahi (1947) noted fever, urticaria and glossitis after a total of only 2 gr. (0·12 gm.) of emetine hydrochloride. Jacovidès (1923) described disordered vision with photophobia, amblyopia and central scotoma. Protein deficiency increases emetine toxicity (Guggenheim and Buechler, 1948).

Nervous Sequelæ. Occasionally peripheral neuritis is seen ;

it is, however, comparatively rare. Thus Brown (1935) noted sixteen cases of polyneuritis among 554 patients treated with emetine hydrochloride, but among the same series of cases there were only four instances of urticaria and one of severe diarrhœa. In West Africa, during the war of 1939–45, among 576 Europeans and 1,868 Africans treated for amœbiasis by emetine hydrochloride and emetine bismuth iodide, only one European developed peripheral neuritis. The symptoms of neuritis are associated with degenerative changes in the nerves, for Young and Tudhope (1926) have produced Wallerian degeneration, more especially at the distal ends of the nerves.

Cardiac Sequelæ. As increase in the pulse rate has been described in man together with lowered blood pressure and a feeling of constriction in the throat and chest, sometimes associated with globus hystericus, considerable attention has been paid to the electrocardiographic changes. The possibility that emetine might exercise a dangerous effect on the circulatory system was first suggested by Levy and Rowntree (1916) as a result of experiments on animals. Maurel (1914a, b), Pick and Wasicky (1916–17), Plumier-Clermont (1919), and Biberfeld (1919) were the first to draw attention to the fall in blood pressure produced by an intravenous injection of emetine. Later Boyd and Scherf (1941) showed that after intravenous injection of emetine into cats and dogs definite changes occur in the electrocardiogram within twenty to thirty seconds. The first change noted is a widening of the ventricular complex accompanied by cardiac dilatation, especially involving the right ventricle. The T wave tends to assume a reciprocal relationship to the initial deflection. Bradycardia and prolongation of atrioventricular conduction time develop regularly but are not pronounced. The appearance of disturbances of conduction in the intact heart after large doses of emetine had already been noted by Epstein (1932), but the most common arrhythmias in cats and dogs are auricular extrasystoles and auricular tachycardias. Alternation of the ventricular complexes is specially marked during the tachycardias. Advanced stages of intoxication are required to produce ventricular extrasystoles, and heart block with dropped beats is encountered only in cats. When the intravenous dose did not exceed 37 mgm. per kgm. the electrocardiographic alterations gradually disappeared within forty-five minutes, cardiac dilatation vanishing in the same time. The action of emetine on the heart muscle can be shown to be cumulative, for much more pronounced effects result from the second or third injection although the normal electrocardiogram has been restored. It has, however, been noted by Hein and Vannotti (1939) that while histological changes may be quite pronounced in the hearts of animals with chronic emetine poisoning, the electrocardiograms may show little change. Tournade and Sarrouy (1935) and Tournade *et al.* (1935) found that the bradycardia and cardiac dilatation following intravenous emetine in dogs were not affected by section of the vagus, nor were they modified by intravenous injection of atropine.

In rabbits, Berman and Leake (1928) noted a predominance of ventricular tachycardias and the frequent appearance of auricular fibrillation. There is, however, a possibility that Berman and Leake misinterpreted their tracings.

In man, Chopra and Sen (1934) noted in one instance a depression of the S—T segment ; however, it is possible that the condition may have been present before the administration of emetine. More recently, careful investigations of the human heart have been made before the beginning of emetine treatment, during its course, and for some time after the last injection. Heilig and Visveswar (1943) studied the electrograms in Indians given emetine in therapeutic doses intramuscularly or intravenously. Many patients had abnormal electrocardiograms on admission to hospital as a result of anæmia and infections : thus of fourteen given intramuscular injections of emetine, 1 gr. (0·06 gm) daily, no less than eleven were abnormal before treatment. At the end of the course eleven of the fourteen exhibited an improvement in their electrocardiograms, two showed no change, and one, previously normal, became abnormal. In patients given emetine intravenously there were frequent electrocardiographic changes within one hour. The T waves in one or more leads were flattened in five cases, the voltage in R was diminished in one or more leads in three cases, and a prolongation of P—Q was seen in two cases. The two cases with delayed conduction occurred after the first injection of emetine. Among fifteen patients who already had considerable myocardial involvement, nine showed a lower T in one or more leads, six showed diminished height " voltage " of R, while one exhibited an increased " voltage." At the end of the course five had improved, one had remained static and nine showed deterioration. Blood pressure changes were noticeable only after intravenous injection, when within ten or fifteen minutes both systolic and diastolic pressures fell 5 to 10 mm. of mercury.

Of seventy-two patients receiving 1 gr. (0·06 gm.) of emetine subcutaneously for ten days, studied by Hardgrove and Smith (1944), thirty-eight showed electrocardiographic change in all limb leads, though lead IV was usually less involved. Q wave changes were not seen : thirty-three had depression of the T waves varying from slight lowering of the amplitude to complete inversion. Ten showed inversion in one or more leads. The auriculoventricular conduction time was increased in seven cases but in only one did it become abnormal, the P—R interval being lengthened to 0·24 seconds, thus showing the first degree of A—V heart block. In one case auricular systoles disappeared and ventricular extra beats appeared as an immediate effect after the injection of emetine. The changes in the electrocardiogram disappeared in a week or two after cessation of treatment.

In otherwise healthy British soldiers Cottrell and Hayward (1945) noted similar changes with emetine hydrochloride injected intramuscularly or emetine bismuth iodide by mouth. Diminution of the T waves occurred in one or more leads in twenty-five of thirty-two cases, while in twelve there was an increase in the P—R interval of from 0·02 to 0·04 seconds among those treated with emetine hydrochloride : seven of eight cases given emetine bsimuth iodide showed diminution of the T waves and prolongation of the P—R interval. The electrocardiograms returned to normal in eight to twelve days after completion of treatment. Effects on the blood pressure and pulse rate were insignificant.

Brown (1935), among 554 patients treated with emetine, observed no untoward symptoms involving the heart, nor were any cardiovascular accidents seen among 2,444 cases in West Africa. Sodeman and Lewis (1945), among seventy-eight patients suffering from amœbic hepatitis, noted only one with severe cardiac symptoms.

It will thus be seen that while emetine does temporarily affect the electrocardiogram, there are no residual effects. It must, however, be remembered that in animals without definite electrocardiographic abnormalities histological changes may be found in the heart muscle.

Other lesions have been noted in animals by Dale and Dobell (1916), Chopra, Ghosh and De (1924), Anderson and Leake (1930), Rinehart and Anderson (1931), and Hein and Vannotti (1939) as the result of toxic doses of emetine. After six or seven injections in rabbits the heart muscle may show cloudy swelling, loss of transverse striation, hyaline degeneration and areas of fibrosis: hyaline changes have also been recorded in the diaphragm. After eight or nine injections the lungs of animals show œdema, congestion and hæmorrhage into the alveoli. Widespread hæmorrhages from injury to capillary endothelium are by no means rare.

Similar lesions have been described in man in fatal cases of emetine poisoning by Bais (1921), Soca (1922), Leibly (1930) and others. In subacute or chronic emetine poisoning death is usually due to respiratory failure. In rats toxicity is increased by a low protein diet.

Nevertheless there is evidence that the indiscriminate use of emetine, and particularly its employment as a diagnostic agent, may not be without danger. The clinical impression of old and cautious tropical practitioners is supported by Dack and Moloshok (1947), who describe nine cases of amœbic dysentery in which toxic cardiac manifestations developed after treatment with emetine hydrochloride in doses varying from 7 to 22 gr. (0·42 to 1·32 gm.). In addition to fatigue, dyspnœa and tachycardia on mild exertion, electrocardiographic abnormalities involving T waves in all leads were noted. The dose of emetine necessary to produce cardiac effects varied in each case, suggesting individual differences in susceptibility and in the rate of excretion of the drug. The time of appearance of the electrocardiographic abnormalities also varied in each case and was often delayed till one or two weeks following the discontinuation of treatment. Thus the absence of electrocardiographic changes cannot be safely utilised as a criterion for the continuation of emetine hydrochloride therapy beyond a certain dose. The electrocardiographic abnormalities when present may be of long duration, often persisting for two months or more ; sometimes the appearance of toxic cardiac effects may be preceded by toxic effects on the neuromuscular or gastro-intestinal systems.

As a rough clinical guide before the onset of the electrocardiographic changes, muscular weakness, tremor, diarrhœa, and abdominal cramps indicate that emetine is toxic to a particular patient.

Preparations of Emetine

It was only after Schaudinn (1903) had identified *Entamœba histolytica* and its significance as a pathogenic agent had been fully demonstrated, that ipecacuanha began to be used on a rational basis as a specific against the parasite. Some discussion then arose as to the relative values of ipecacuanha with and without emetine in the treatment of amœbiasis. Vedder (1911 and 1912) investigated the question by studying the effects of emetine *in vitro* on an amœba of the *Limax* type and suggested the use of the alkaloid in amœbiasis in preference to the crude ipecacuanha. Rogers (1912), in India, then showed unequivocally that emetine hydrochloride had a specific action in curing amœbic dysentery and liver abscess.

Emetine hydrochloride has been administered by subcutaneous, intravenous and intramuscular injection, by the rectum, and by mouth. The intramuscular route is preferred to the subcutaneous route as it causes less irritation and pain. German physicians, following Petzetakis (1924), for long preferred the intravenous route, despite the liability to cause nausea and vomiting. Emetine injections *per rectum* are extremely irritating and painful. Recently emetine hydrochloride has been given orally by Shrapnel *et al.* (1946) in enteric-coated tablets each containing $\frac{1}{3}$ gr. ; the patients, aged from two to fifty-six years, were treated as follows : seven were given 1 gr. for twelve days ; two patients vomited, possibly as a result of defective tablets, and some complained of slight diarrhœa, but there was no tenesmus ; one patient remained positive ; six cases were given 2 gr. daily for six days : vomiting again occurred in two, and only four became negative ; among four small children there was one failure. Of eighteen patients examined from one to seven months later thirteen were cured while five had amœbæ in the stools, though only one had symptoms. Shrapnel (1947) records the results in a further series of twenty-nine adults and five children who were examined two to nine months after their original treatment. Clinical cure was obtained in all cases but parasitological cure failed in one adult. It should be noted that in Egypt, during the 1914–18 war, Wenyon and O'Connor (1917) obtained the greatest number of permanent cures by giving 1 gr. each morning subcutaneously for twelve days and 0·5 gr. in capsule form each evening by mouth. This dose is possibly too high.

Emetine bismuth iodide was introduced by DuMez (1915), a double iodide of emetine and mercury having previously been employed in India by Walsh (1891). In 1916 Dale used emetine bismuth iodide in the treatment of ten patients with amœbic dysentery which had not been eradicated by previous injections of emetine hydrochloride. Six patients were cured, two continued to show evidence of persisting infection and two were unable to withstand the full course owing to nausea, vomiting and diarrhœa. Low and Dobell (1916) also used the drug in three cases all of which were cured : they believed that emetine bismuth iodide was far more efficacious than emetine by injection in sterilising the gut infections of latent cases. Nevertheless many failures have been reported with emetine bismuth iodide, either because the symptoms which it produces render a full course impossible or, far more commonly, because the coating of the tablets is so hard that they fail to disintegrate in the intestine. A further difficulty noted in hot climates with a high humidity is that if a bottle of tablets is uncorked its contents very readily become converted into a glutinous mass.

It is generally considered to be inadvisable to give emetine bismuth iodide by mouth at the same time as emetine hydrochloride is being given by subcutaneous injection.

Auremetine, introduced by Willmore and Martindale (1926), is still sometimes used because hypodermically it is thought to be less depressing than emetine ; it can be given by mouth. It is a combination of the periodides of emetine and auramine (tetramethyldiaminodiphenyl-ketoniminehydrochloride) with the approximate composition of :—

Emetine	28 per cent.
Auramine	16 ,, ,,
Iodine	56 ,, ,,

It is insoluble in water but is slowly split up in the intestine with the liberation of emetine and auramine.

Quinoline Derivatives

As emetine is an *iso*quinoline derivative it is natural that other compounds of this group should have been tested for amœbicidal action. Child and Pyman (1929) prepared seven derivatives with two *iso*quinoline rings connected by a single carbon chain where the *iso*quinoline rings were substituted in the same way as emetine. *In vitro* these compounds failed to inhibit *Entamœba histolytica* in dilutions lower than 1 in 5,000. David *et al.* (1931 and 1933) further investigated eleven halogenated oxyquinolines, and in addition to chiniofon, which had been originally prescribed for amœbic dysentery by Mühlens and Menk (1921), they reported on the discovery of two other new quinoline compounds now known as vioform and diodoquin, both of which have found a place in the treatment of amœbic dysentery. Halogenation of oxyquinoline increases toxicity, while increased halogenation of the oxyquinoline and increase in the atomic weight of the halogen gives greater action on *Balantidium coli* but not on *Entamœba histolytica in vitro.*

The quinoline compounds of chemotherapeutic interest are: 7-iodo-8-hydroxyquinoline-5-sulphonic acid, mixed with approximately 20 per cent. of sodium bicarbonate (chiniofon B.P., yatren, loretin, quinoxyl, quinosulphan, mixiod, anayodin); 5-chloro-7-iodo-8-hydroxyquinoline (vioform N.N.R., enterovioform, ambisyd enterochin, quinambicide); 5, 7-diiodo-8-hydroxyquinoline (diodoquin, dihaloquin, amœbindon). Chiniofon contains not less than 26·5 or more than 29 per cent. of iodine; vioform, 40 per cent. of iodine and 12 per cent. of chlorine, while diodoquin is said to contain not less than 60·5 per cent. or more than 64 per cent. of iodine. In India, Pal *et al.* (1944) prepared iodochlorohydroxyquinoline, which differs slightly in its chlorine content from that described in "New and Non-Official Remedies, 1941": a study of a number of preparations of vioform showed that the percentage of iodine varied from 38·66 to 41·83, the percentage of chlorine from 10·99 to 17·02.

Chiniofon has now found an established place in the treatment of amœbic dysentery. It can be given both by mouth or per rectum, but it must not be injected intravenously, owing to its liability to give rise to degenerative changes in the renal tubules and, more especially, in the liver (Dyckerhoff, 1935).

By incorporating radioactive iodine into the chiniofon molecule, Albright *et al.* (1947) have studied its metabolism. When chiniofon is given by mouth, absorption regularly occurs, averaging 12·9 per cent. of the drug given. The peak blood level occurs about two hours after administration ; the bulk of the absorbed drug is excreted in the urine in the first twelve hours, the remainder in forty-eight hours. Chiniofon is partly broken down after absorption, iodine being split off. During the initial period of observation the drug is excreted 82 per cent. (average) intact, while at the end of observation only 27 per cent. is intact. For the entire forty-eight-hour period 58·6 per cent. of the drug excreted in the urine is intact chiniofon, the balance being free iodide and organic residue. A portion of the free iodide fraction is detectable qualitatively in the thyroid gland.

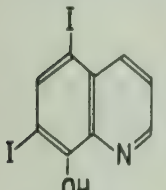
The unabsorbed portion of the drug is eliminated in the fæces, from five to seven evacuations being necessary to remove it all. With the doses ordinarily administered, blood levels of clinical significance are not attained.

Vioform can be given only by mouth. It is almost insoluble in water, while the hydrochloride, which is soluble in water, is irritating to mucous membranes in dilutions of 1 in 500 ; emulsions of the insoluble compound also irritate the lower bowel. Investigations by David *et al.* (1933), in America, and by Pal *et al.* (1944), in India, show that of seventy-one cases fifty-seven were cured by 0·25 gm. three times a day for ten days.

With diodoquin, earlier experiments in dogs suggested that the greater part of the compound could be recovered from the fæces, the iodine being still united with the quinoline nucleus. David *et al.* (1944) found, however, that absorption undoubtedly does occur both in animals and in man : when volunteers were given doses of 0·21 to 0·25 gm. thrice daily for ten days the iodine concentration in the blood, normally 5 to 20μgm. per 100 ml., rose to an average of 172 μgm., the corresponding figure for vioform being 223 μgm. per 100 ml. The absorption of diodoquin was more irregular than that of vioform, and in one instance the iodine content of the blood was 437·25 μgm. per 100 ml. Some of the volunteers complained of gastro-intestinal discomfort, and a few developed pruritus ani, usually on the fourth or fifth day of administration. To allay abdominal discomfort, phenobarbitone 1 gr. (65 mgm.) may be given one hour before each enema. In view of the irregularity of absorption, the number of deaths in experimental animals is not proportional to the dose, so that no LD 50 can be obtained : from 20 to 40 per cent. of guinea-pigs die, however, after doses ranging from 50 to 2,000 mgm. per kgm. of body weight. With vioform the LD 50 of a single oral dose is 175 mgm. per kgm. in guinea-pigs and approximately 400 mgm. in kittens. In animals which die after fatal doses of either vioform or diodoquin there is extensive necrosis of the liver cells (David *et al.*, 1933 ; David *et al.*, 1944). As diodoquin is opaque to X-rays it can be demonstrated that there is a very even distribution of the drug throughout the large intestine. Tenney (1936) and Craig (1937) obtained good results in the treatment of human amœbiasis and of experimental infections in dogs, but no thorough comparison with emetine and chiniofon was made. D'Antoni (1942 and 1943) found that for adults three tablets, each of 3·2 gr. (192 mgm.), three times a day, was insufficient if given for only ten days ; the course was therefore continued for a further ten days : children under ten years of age were given two-thirds the adult dose.

The most extensive series of patients is that reported by Morton (1945). One series of twenty-six cases was treated by diodoquin by mouth, three tablets of 3·2 gr. three times a day for twenty days : twenty were cured, though of these three had been diagnosed on sigmoidoscopic findings only, four were resistant and two relapsed. In a second series thirty-nine cases were treated by diodoquin for twenty days while for the last ten days they also received chiniofon retention enemata : thirty were cured, though of these eight had been originally diagnosed on sigmoidoscopic findings only, two patients were resistant and seven relapsed.

Thus of sixty-five patients fifty were cured, fifteen were still infected. These results may be compared with thirty-seven treated either with injections of emetine hydrochloride and diodoquin by mouth (thirteen cases) or with emetine bismuth iodide by mouth, retention enemata of 250 ml. of 2·5 per cent. chiniofon for ten days and carbarsone 4 gr. (240 mgm.) twice daily for ten days (twenty-four cases).



Diodoquin

Of these two combined series twenty-seven were cured, three were resistant and seven relapsed : thus there was little difference in the cure rate between those who did and those who did not receive emetine. Rickards (1949) cured 91 of 127 consecutive cases : there were few indications for using diodoquin alone in early treatment. Silverman (1937) gave somewhat large doses of diodoquin, twelve tablets, each of 3·2 gr. (192 mgm.), being administered daily for twenty days ; no toxic reactions were observed among twenty-five patients with acute and chronic amœbic dysentery. Hummel (1939) treated twenty patients with nine tablets a day for twenty days without any evidence of toxicity, and D'Antoni (1943) did not note any toxic reactions in eighty-four cases treated with diodoquin. Further observations on the treatment of amœbic dysentery with diodoquin in comparison with other drugs are required. While for the most part diodoquin has little toxicity, the pruritus ani, previously referred to, may persist for some days after the end of treatment : occasionally petechial hæmorrhages may appear in the rectal mucosa (Morton, 1945). Silverman and Leslie (1945) reported two patients with furunculosis, and a third developed a sore throat with a blotchy erythema over the whole body. Morton (1945) had two cases where, on the fourteenth day of treatment, severe abdominal pain, headache and diarrhœa set in : these symptoms were relieved by injections of emetine hydrochloride. Rickards (1949) among 127 cases noted toxic effects in sixteen : eleven had pruritus ani, one had mild furunculosis, two nausea, vomiting and diarrhœa, three headache, and two mental depression. A slight enlargement of the thyroid gland is extremely common as a result of taking diodoquin. Since *in vitro* none of the oxyquinolines has a marked amœbicidal action, it is possible that the curative action may be due to the destruction or inhibition of certain of the bacteria on which the amœbæ live. *In vivo* during treatment with these drugs increased fragility of the vegetative forms is observed. Cytological changes are not uncommon: karyorrhexis is present and the karyosome cannot be observed in all cases; the nuclear ring becomes swollen and then disrupts. The nuclei of cysts show similar changes, vacuoles are often seen in the cysts and mature forms are rare (Young, 1946).

Observations by Manson-Bahr and Muggleton (1948) suggest that diodoquin is perfectly capable alone of eradicating the parasites from patients who, without symptoms, are expelling cysts but that it cannot destroy amœbæ which have penetrated into the wall of the gut. Parnell (1948) finds diodoquin by mouth as effective as chiniofon enemata.

The use of retention enematas which cause much discomfort and distress might well be abolished.

PENICILLIN AND THE SULPHONAMIDES

It is now generally agreed that neither penicillin nor the sulphonamides has any direct action on *Entamœba histolytica:* nevertheless there is considerable evidence to show that by their action either on intercurrent bacterial infections of the intestinal wall or on the bacterial flora in the intestine these compounds may bring about clinical improvement in cases of long-standing amœbic dysentery. The importance of the bacterial flora in amœbic dysentery was emphasised by Acton and Knowles (1928), who attempted to modify it by using vaccines. Hargreaves (1944 and 1945) found that penicillin had a remarkable effect on a patient almost moribund from amœbic dysentery: an initial intramuscular dose of 100,000 units was followed by 33,000 units three-hourly up to a total of just over one million units. The results were dramatic, as in twenty-four hours after starting treatment the patient was apyrexial and free from pain. Ten other patients were then treated with penicillin and sulphasuxidine with very considerable improvement in their symptoms, but with no evidence of any direct action on the amœbæ. However, after treatment with penicillin and sulphasuxidine, subsequent treatment with emetine bismuth iodide brought about disappearance of the amœbæ. Willmore (1944) also obtained good results with penicillin.

The value of penicillin in chronic amœbiasis has since been confirmed by many workers (Blanc and Siguier, 1946 ; Benhamou *et al.*, 1947). Seyberlich (1948) finds that penicillin given per rectum is of value when 500,000 units are administered in two days at the same time as specific therapy. Some observers believe that all cases of amœbic dysentery, acute or chronic, should be given two million units as part of the routine course. This has been combined with 65 gm. of sulphasuxidine, emetine bismuth iodide by mouth and quinoxyl enemata for twelve days, and carbarsone for ten days (Wright and Coombes, 1948). Procaine penicillin is now being used : 150,000 units twice a day for four days appears to be satisfactory.

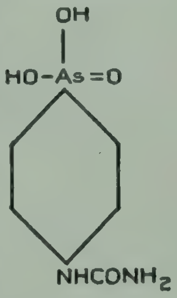
Halawani *et al.* (1945) noted that, though cysts are not affected by sulphaguanidine, vegetative forms tend to become encysted, combined courses of emetine and sulphaguanidine giving better results than emetine alone. Payne (1945a and b), while confirming the good effects of penicillin and sulphasuxidine, believed that even sulphapyridine may be of use. Coghill (1945) recommended sulphonamides by mouth in both acute and chronic cases of amœbic dysentery. The removal of secondary bacterial infection from the intestinal wall and the possible alteration of the bacterial flora of the intestine by penicillin and either sulphasuxidine or sulphaguanidine marks a definite advance in the chemotherapy of amœbiasis.

Less dramatic results were observed in many theatres of war in patients treated with sulphaguanidine or sulphasuxidine to a total of 80 gm. There is evidence that penicillin and the sulphonamides may act synergistically on *Bact. coli, Streptococcus fæcalis* and the paracolon bacillus, thus allowing effective bacteristatic concentrations to persist in the gut for considerable periods. Sulphasuxidine without penicillin is of little value in acute cases. Failure to show improvement after penicillin and sulphonamides may be due to the presence of resistant bacteria or of active penicillinase producers. Good results have been claimed with aureomycin, 500 mgm. four times a day for four days. Streptomycin and penicillin exert a synergistic action on *E. histolytica* in cultures (Seneca *et al.*, 1949).

ARSENICAL COMPOUNDS

Although quinquevalent arsenicals are undoubtedly of value in amœbiasis, it is now generally agreed that they should not be relied on alone to eradicate amœbic infection either of the bowel or of the liver.

The first arsenical to be introduced in the chemotherapy of amœbiasis was acetarsol; later tréparsol, 3-formylamino-4-hydroxyphenylarsonic acid, was introduced by Flandin (1924). Both these compounds are now far less commonly used than 4-carbamidophenylarsonic acid (carbarsone N.N.R., amibiarson, aldarsone, kutan), which, when anhydrous, contains 28·85 per cent. of arsenic. It was first prepared by Ehrlich and Bertheim (1909) and is a white crystalline solid, stable in air, melting at 174° C. and practically insoluble in water, though it dissolves readily in alkaline aqueous solution.



It was first examined for toxicity by Chen *et al.* (1930) and used in the treatment of amœbiasis in man by Reed *et al.* (1932) and Reed and Johnstone (1934). Of 175 persons with amœbiasis only four are said to have continued to show amœbæ in the stools after a single course of treatment. This treatment consisted of 75 mgm. per kgm. of body weight in divided doses spread over a period of at least ten days: in the case of the average adult, 0·25 gm. was given by mouth twice daily for ten days. The smallest dose which proved effective in eradicating amœbæ was 29 mgm. per kgm. of body weight. More recent investigators have preferred to combine emetine and carbarsone or carbarsone and chiniofon. Thus Mateer *et al.* (1940) claim to have cured 101 out of 104 patients by doses of 0·25 gm. of carbarsone for ten days, combined with 250 ml. of 2·5 per cent. chiniofon in the form of retention enemata every other day. Patients were observed for follow-up periods of from six months to two and a half years.

Recently the tervalent analogue of carbarsone, *p*-carbamidophenylarsenous oxide, has been found to have greater amœbicidal activity than carbarsone both *in vivo* and *in vitro* (Anderson and Hansen, 1947). Its pharmacological properties have been studied by Anderson, Bond and Abreu (1946). The arsenic content is 32 per cent., while the LD 50 by mouth for rats is 510 ± 40 mgm. per kgm. of body weight. The administration of large amounts causes gastric irritation and hæmorrhage, but these changes are less marked if equal parts of propylene glycol and water are used as solvents. Arsenic is stored in the body in largest amount, in descending order, in liver, kidneys, lungs, and spleen. Rabbits tolerated oral doses of 75 mgm. per kgm. of body weight in enteric-coated tablets for thirty-four days : they died if given 20 to 40 mgm. per kgm. of body weight intravenously. Monkeys tolerated oral doses of 11, 17, and 27 mgm. per kgm. in enteric-coated tablets for forty-five days without impairment of hepatic or renal function or loss of weight. In man, daily doses of 30 and 60 mgm. in enteric-coated tablets were tolerated for ten days without evidence of dysfunction. Two other tervalent arsenicals, containing sulphur, were found by Anderson *et al.* (1947a and b) to be effective in monkeys infected with *E. histolytica.* Phenyl urea *p*-di-(carboxy methyl)-thioarsenite is less toxic than carbarsone, and monkeys given seven daily doses of 25 mgm. per kgm. showed no ill effects, as well as being freed from infection : 4-carbamido-phenyl-di-(1′-carboxy-phenyl-2′)-thioarsenite was equally effective in monkeys in the same dosage when given over a period of eight days.

As with other arsenoxides, the arsenic of carbarsone oxide and its dithio derivatives are bound by tissues roughly in proportion to the toxicity of each drug. The concentration of arsenic in bile and intestinal tissues after injection of phenylarsenoxides appears to be greater than after proportional doses of arsonic acids (Chance *et al.*, 1945 ; Anderson *et al.*, 1947b).

Anderson and his colleagues (1949) have recently used *p*-carbamidophenyl-bis (carboxymethylmercapto) arsine as well as *p*-carbamidophenyl-bis (2-carboxyphenylmercapto) arsine. Of thirty-eight patients given the first compound, thirty-three were promptly cured and have remained free from infection : of forty-four patients given the second, forty-one were cleared. The dosage by mouth varied from 3·0 gm. in ten days to 7·2 gm. in twenty-four days. In addition, thirteen patients were given retention enemata of 3·0 to 6·0 gm. in six days. No cutaneous reactions occurred but twelve patients exhibited nausea or vomiting after 200 mgm.

doses of either thioarsenite. Coating of the tablets with phenyl salicylate permitted completion of therapy in all but one instance. Three patients, one of whom was also given emetime, were apparently cured of amœbic hepatitis by thioarsenites. Two patients have been cured of infection due to *Balantidium coli*. It would thus appear that the substitution of sulphydril groups into the carbarsone molecule provides more active and more widely distributed amœbicidal compounds.

Oxophenarsine, which *in vitro* has a slight amœbicidal action (Anderson and Chuan, 1944), would appear to have no advantage over carbarsone.

The action of the arsenoxides on amœbæ is in accordance with the view put forward by Voegtlin *et al.* (1923) that protozoa as a group contain, and are dependent upon, smaller absolute amounts of —SH groups than mammalian tissue cells.

Other Compounds used in Amœbic Dysentery

Subtilin, *in vitro*, has been shown by Anderson *et al.* (1946) to have an *in vitro* action on *E. histolytica*.

Anderson *et al.* (1947a) found that 1-(2′, 4′, 5′-trimethylbenzyl)-2-aminoethane dihydrochloride had some action in bringing about a temporary clearance of the stools in monkeys when given by mouth for seven days in doses of 30 mgm. per kgm. of body weight. *In vitro* it was less active than emetine. An *iso*thiourea, 1, 3-di*iso*butyl-amino-2-isothiourea propanol hydrochloride caused only a temporary clearance of the stools.

Amœbic cysts are apparently able to resist high concentrations of streptomycin, but vegetative forms are not inhibited by a concentration of 1 in 10,000 (Balamuth and Wieboldt, 1946).

Amœbæ die only when an antibiotic causes bacteriostasis and thus changes oxidation-reduction potentials of bacteria from negative to positive levels (Bradin and Hansen, 1949).

Eusol, bismuth subnitrate and silver nitrate should now be discarded in the treatment of amœbiasis.

Brief reference may be made to the use of sodium tetraiodophthalein (opacol) in South Africa, where Alexander *et al.* (1944) have treated thirty children and three adults : the dye is absorbed from the intestine, secreted by the liver, and excreted by the bile.

A half bottle is given at night to children under six years of age; a full bottle is given to those above six years: next morning a salt purge is taken. It is claimed that twenty-two of thirty patients were cured. Further investigations are required.

The seeds of *Brucea javanica* and *B. sumatrana*, under the name of ya tan tzu or kô-sam, have been used in China for at least 180 years in the treatment of dysentery. Although some forty years ago a pharmaceutical preparation of kô-sam was available, it seems essential for success to prescribe the whole seed. Good results have been claimed by Liu (1937) and Wu (1943). The course of treatment is as follows: on the first, third, and fifth days twenty seeds in their capsules are given by mouth three times a day, and on the second, fourth and sixth days twenty seeds are soaked for two hours in 200 ml. of 1 per cent. sodium bicarbonate and given as an enema to be retained after a washout. Wu treated twenty-five patients of whom ten were acute and fifteen chronic; nineteen cases cleared in two to five days and *Entamœba histolytica* could no longer be found in the stools: two patients relapsed three and six weeks after discharge from hospital; one of these was cured by a further course of ya tan tzu. Toxic effects were negligible; eight patients complained of nausea and four vomited; a few had abdominal discomfort or actual pain, but these symptoms may have been due to the dysenteric lesions. Very little is yet known of the active principle of ya tan tzu, but Power and Lees (1903) failed to find any evidence of an alkaloid. All that was found were two bitter principles containing carbon, hydrogen and oxygen. There was no evidence that these were glycosides and neither was related to quassin. Cheng *et al.* (1944) examined the expressed oil, a volatile oil distilled by steam from the expressed oil, and ether, chloroform, and alcoholic extracts from the oil-free portion. These fractions were given by stomach tube and by subcutaneous and intravenous injection to rabbits. The oil-free portion is toxic and in doses of 0·19 gm. per kgm. of body weight caused nausea, vomiting, blood in the stools and vomit, convulsions and death. The alcoholic extract caused a temporary fall in blood pressure while the volatile oil produced slight gastro-intestinal irritation; the ether extract and the oily portion were non-toxic. The histological changes caused by the oil-free portion are hæmorrhages in the stomach and intestines, fatty degeneration of the liver, cloudy swelling of the cells of the convoluted tubules of the kidney and hæmorrhage and congestion in the spleen. No tests have yet been made to determine which, if any, is the alleged amœbicidal component of ya tan tzu.

During the war of 1939–45 the Japanese used the roots of *Stephania rotunda* which grows in Indo-China ; there is no evidence that it is of value. In France the total alkaloids of Kurchi bark, long used in India, have once more come into favour.

Chloroquine has been shown to have some value as an amœbicide in cases of dysentery and of hepatitis. Conan (1948) found that of twenty-eight patients with dysentery fifteen became free from parasites for from two to eight months while thirteen showed no change ; six patients with amœbic hepatitis cleared up rapidly and remained free from symptoms. The dose schedule employed was 0·3 gm. of the base twice daily for two days followed by 0·3 gm. once daily for from twelve to nineteen days. No toxic symptoms were noted except in two patients who complained of mild gastric uneasiness.

The Question of Emetine-resistant Entamœbæ

During the war of 1914–18 failure to cure cases of amœbic dysentery was frequently attributed to the acquirement of emetine fastness by certain strains of *Entamœba histolytica.* A similar explanation has been advanced during the second World War to account for the small percentage of patients who resist course after course of emetine or emetine bismuth iodide (Adams, 1944). The evidence that *Entamœba histolytica* can become resistant to emetine is still doubtful. It is common experience that those who have failed to respond to emetine may quite suddenly clear up or, on the other hand, a patient who has never received treatment may show no amelioration after a routine course of emetine, vegetative forms reappearing in the stools a short time after the cessation of emetine therapy.

Under laboratory conditions there have been two claims to have produced emetine-resistant amœbæ, those of Halawani (1930) and of Bonnin and Aretas (1938). Halawani's conclusions have been criticised as fallacious, because he used solid-liquid media with which in chemotherapeutic experiments the experimental error is greater than the effects supposed to be produced. As shown by Laidlaw, Dobell and Bishop (1928) the medium devised by Boeck and Drbohlav (1925), consisting as it does of solid and fluid, is unsuitable for the chemotherapeutic testing of alkaloids. On incubation a large but variable proportion of the emetine added to the serum passes into the coagulated egg so that, although a known amount of alkaloid may have been added to a given culture, the actual concentration present in the liquid in a condition to act on the amœbæ rapidly becomes unknown and uncertain. Laidlaw and his colleagues devised a fluid medium and found that it was essential to buffer it, otherwise, since the final pH of the cultures varied, irregular results were obtained. In addition, Halawani used a liquid medium, dilute egg white in Ringer's solution, in which, according to Dobell (1945), entamœbæ cannot multiply but survive only for a variable time depending upon the pH of the medium, the bacterial flora and other factors, all of which Halawani ignored. Bonnin and Aretas (1938), who also claim to have produced emetine-resistant strains *in vitro,* used similarly unsatisfactory methods. On the other hand, St. John (1933) entirely failed to induce resistance to emetine, and Dobell (1945), who now grows *Entamœba histolytica* in a fluid medium with one known strain of bacterium, has also been unable to produce any evidence of emetine resistance, the amœbæ at pH 7·2 succumbing in three or four days' time to concentrations of even less than 1 part of emetine hydrochloride in 5,000,000.

While there is no theoretical reason why emetine-resistant strains of *Entamœbæ histolytica* should not be produced, the evidence, both clinical and experimental, so far available does not warrant the conclusion that such strains have been produced. It is noticeable that alkaloids as a class appear to be less liable to produce resistance than other chemical compounds, as witness the rarity of quinine-resistant strains of malarial plasmodia. It must, however, be noted that even in the case of trypanosomes some strains are far more easily rendered resistant than others: in amœbiasis the number of strains of *Entamœba histolytica* on which full investigations have been carried out is still very small.

Although experimental evidence of emetine resistance is unsatisfactory, a strain of *E. histolytica* obtained from a patient who has frequently relapsed required ten times the dosage of emetine to inhibit infection in rats than that usually required to inhibit infection. The patient, in addition, continued to pass active amœbæ when treated daily with 1 gr. (0·065 gm.) of emetine hydrochloride parenterally and 3 gr. (0·195 gm.) of emetine bismuth iodide (Murgatroyd, 1947). On the other hand, a strain which is extremely sensitive to emetine has been isolated from a patient who had failed to react to numerous courses of emetine (Goodwin, 1947).

Chemoprophylaxis

Dale (1916), when first using emetine bismuth iodide therapeutically, suggested that this compound might be used prophylactically. This suggestion has not been adopted. Later, Craig (1940) proposed that diodoquin might prove of prophylactic value, seven tablets of 0·21 gm. (3·2 gr.) being taken daily for twenty days. Such a course may be of value for persons on short visits to endemic areas, but is hardly feasible for residents who would have to take repeated courses. As has been pointed out, diodoquin is potentially toxic as a result of the irregularity with which it is absorbed and no extensive use has been made of this compound as a prophylactic. A prophylactic compound against *Entamœba histolytica* should obviously be non-irritant and non-toxic if absorbed from the intestine. The whole question of prophylaxis in amœbiasis is bound up with individual susceptibility of the tissues to the invasive action of *E. histolytica.* Thus the carrier rate for cysts is about the same in Australia as in New Guinea, but in the former country amœbic dysentery is rare, in the latter common. There are undoubtedly also many individuals who after many years' residence in the tropics have never suffered from amœbiasis while others develop symptoms within a few weeks of reaching the tropics. A factor which is possibly of great importance in determining who will or will not suffer from amœbiasis is the character of the intestinal bacteria, and the changes, qualitative and quantitive, which these bacteria exhibit under tropical conditions, Of this factor little or nothing is known.

The Testing of Amœbicidal Compounds

The slow progress of research into new amœbicidal compounds is in part due to the lack of suitable experimental techniques.

Cultivation of amœbæ in media containing a liquid and a solid phase is unsatisfactory for the chemotherapeutic testing of drugs owing to the unequal distribution of the drug in the liquid and solid phase. Various media (Laidlaw *et al.*, 1928 ; Frye and Meleney, 1939 ; Felsenfeld and Young, 1945 ; Emerson and Hansen, 1946 ; Jones, 1946) have been suggested.

A considerable number of attempts has been made to develop *in vitro* methods for the quantitative estimation of amœbicidal activity. Owing to the large number of variables it is now generally agreed the results cannot be regarded as of more than qualitative value and any correlation between *in vitro* and *in vivo* activity is at present highly speculative (Rawson and Hitchcock, 1947). *In vitro* experiments demonstrate very clearly, however, that the action of emetine on amœbæ is a direct one, but *in vitro* experiments must obviously be supplemented by chemotherapeutic experiments in animals. It has long been known that kittens, puppies, and monkeys, can be infected *per os* or *per rectum.* The disease set up, however, in young animals is an extremely acute one and there are also difficulties in keeping large numbers of infected kittens or puppies in laboratories owing to their liability to intercurrent virus infections. Jones (1946) has shown that three- to four-week-old rats can be infected *per rectum* or, at laparotomy, by intracæcal injection of dysenteric material obtained either from infected kittens or from concentrated cultures mixed with mucin ; the number of positive results is greater after intracæcal injection. At necropsy the degree of infection in rats varies ; in some the cæcum is very inflamed, hyperæmic and thickened, with definite amœbic ulceration of the wall, while in others the amœbæ are present in the lumen but no pathological lesions are detectable. A scheme of assessment is necessary for determining the degree of amœbic infection in rats and for calculating the " average degree of infection " for a group. The two main factors influencing the average degree of infection in a group of rats are (*a*) the weight of the rat—those weighing 20 to 33 gm. giving the best results ; and (*b*) the duration of the infection, which is at its height after three to six days, though ulceration can be detected twenty-four hours after injection. The infection gradually disappears from the majority of rats in from seven to twenty-eight days after injection of amœbæ. Only a small proportion of rats show clinical signs of infection, such as diarrhœa with mucus and blood, but some rats, which appear to be normal, reveal cæcal ulceration at necropsy. Cysts of *E. histolytica* have occasionally been seen after fourteen to twenty-eight days, but the examination of fæces does not provide a reliable method for the diagnosis of the infection.

Experimentally infected rats are employed for therapeutic tests of amœbicidal properties of various compounds, using the following standard procedure. Sixty rats, aged three to four weeks and weighing 20 to 33 gm., are divided according to weight into five groups. A concentrated suspension of *E. histolytica* is made by pooling and centrifuging the deposits of twelve Roux-bottle cultures grown for two days at 37° C., and mixing 8 ml. of the suspension with an equal volume of 10 per cent. mucin in water. The mixture, containing about 1,000,000 amœbæ per ml., is kept in a water-bath at 37° C., and rats anæsthetised with ether are injected after laparotomy with 0·2 ml. of the suspension. One group serves as a control, while the remaining groups are dosed with the drug by subcutaneous or oral injections twenty-four hours after the operation. The rats are killed five days later, the cæcum being examined macroscopically, and its contents microscopically, for the presence and concentration of amœbæ. Each rat is then awarded a " score " of from 0 to 5, according to the condition of the cæcum. The mean score (average degree of infection) for each group is determined and tested for significant difference from the mean of the control group by the usual statistical methods. Active compounds give a significant difference from the controls ($P = 0{\cdot}05$ or less). A value of P of 0·05 to 0·2 is regarded as indicating slight activity, and a value of more than 0·2 indicates low activity.

The effects of drugs studied by this method are as follows :—

THE EFFECT OF DRUGS ON EXPERIMENTAL AMŒBIASIS IN RATS (Jones, 1947)

	Dose, mgm/kgm. orally 24 hours after operation.		Significance of treatment. + = P < 0·01. ± = P, 0·01 – 00·5, – = P > 0·5.				
	Emetine.	Chiniofon and other compounds.	Emetine.	Chiniofon.	Acetarsol.	Carbarsone.	Diodoquin.
Single dose therapy.	20	1,000	+	+	+	+	±
	10	500	+	+	–	–	–
	5	250	+	±	–	–	–
	2·5	125	+	±	...	...	...
	2·0	100	–	–	...	...	...
	Orally 24, 30, 48, 54 and 72 hours after operation.						
	5	1,000	+	+	+	+	+
	2·5	500	+	+	±	+	+
	1·25	250	±	–	–	–	–
	0·62	125	±	–	...	–	...

It will be noted that when given as a single dose diodoquin was hardly effective ; when repeated, its action was more marked.

Neither carbarsone nor emetine has any effect on *E. muris* in rats : a result of using rats infested with this parasite may be to invalidate chemotherapeutic experiments (Neal, 1949). Goodson *et al.* (1948a, b) and Goodwin *et al.* (1948), using methods of assay *in vitro* and *in vivo* in young rats, have studied the effects of several series of secondary diamines formally related to emetine. Bis (β-3 : 4-dimethoxyphenylethylamino)-alkanes in which the hydrocarbon chain contained six to ten carbon atoms were active, but the corresponding 4-monomethoxy compounds were less active : bis (β-phenylethylamino) alkanes also showed activity which was increased by the introduction of chlorine into the *ortho-* or *para*-position of the benzene ring. The *ortho* compound was most active but also rather toxic. Bis (phenylalkylamino) alkanes with either a greater or fewer number of carbon atoms between the nucleus and the amino group were less effective. Bis (alkylamino) alkanes containing seven or eight carbon atoms in the alkyl groups and six to ten carbon atoms in the connecting chain also showed activity which was higher *in vivo* than that of the bis (β-phenylethylamino) alkane series. Results at low dose levels were, however, erratic.

Treatment

It is now generally realised that by employing any one of the amœbicidal drugs here discussed alone it is not possible to cure any great percentage of cases, but by a judicious combination of these drugs and by careful attention to the details of treatment it is possible to eradicate *Entamœba histolytica* in practically every case. It is, however, a matter of considerable importance that treatment of amœbic dysentery should begin as soon as possible after the onset of symptoms ; a correct diagnosis is therefore of considerable moment. Early in the war of 1914–18 much valuable time was wasted because of the belief that almost all patients with dysentery were necessarily suffering from amœbiasis ; with greater skill in clinical pathology this fallacy has now been eliminated and treatment can usually be commenced at an earlier stage. The course of treatment will depend, however, on whether the patient is suffering from :—

(1) Acute symptoms with vegetative *Entamœba histolytica* in the stools.

(2) Mild or no symptoms with the passage of cysts but with no evidence of hepatitis.

(3) Repeated attacks of dysentery that have resisted routine treatment with emetine hydrochloride, emetine bismuth iodide, and chiniofon.

(4) Hepatitis.

(5) Amœbic abscess.

For patients with acute dysenteric symptoms and vegetative entamœbæ in the stools, emetine hydrochloride, 0·06 gm. (1 gr.) daily for four to six days by intramuscular injection will usually be sufficient to control symptoms. The patient must be kept at rest in bed. Emetine bismuth iodide is then given, without an interval, in doses by mouth of 0·2 gm. (3 gr.) daily for twelve consecutive days, care being taken to examine the stools to see that they are blackened by the drug, an indication that the tablets are disintegrating in the bowel: emetine bismuth iodide is preferable to auremetine. At the same time as the oral emetine bismuth iodide, daily enemata of chiniofon should be given, following an alkaline washout with sodium bicarbonate solution. For the first six days 200 ml. of a 2·5 per cent. solution of chiniofon should be administered followed for the remainder of the course by 300 ml. of 5 per cent. solution if the weaker preparation has been well tolerated. It is essential, however, to see that the chiniofon is retained for at least six hours, and to ensure this retention the patient must be kept at rest for this period. Thereafter treatment may be completed either by giving 0·25 gm. of carbarsone or acetarsol twice daily for twelve days; diodoquin is, however, preferable and may be given 0·2 gm. (8·2 gr.) three times a day for twenty days. It is only during the third stage of treatment that the patient is allowed up.

COMPARATIVE EFFECTS OF DIFFERENT METHODS OF TREATMENT (Rail, 1947).

Series.	Course.	Number of case.	Relapses (within twenty-one days).
I.	Emetine hydrochloride 1 gr. (65 mgm.) s.c. for eight days: four days' rest and 1 gr. for four days: carbarsone 0·25 gm. daily for ten days, beginning on the ninth day.	50	9
II.	Kurchi bismuth iodide 10 gr. (0·65 gm.) daily: carbarsone 0·25 gm., both daily for ten days.	50	24
III.	Vioform 0·5 gm. daily for twelve days and concurrently carbarsone 0·25 gm. for ten days.	50	11
IV.	As in Series I. Sulphapyridine 2 gm. initially and 1 gm. six-hourly to a total of 13 gm.	50	3
V.	Emetine bismuth iodide 65 mgm. the first night and 130 mgm. for nine days. Chiniofon 2·5 per cent. retention enemata for ten days.	50	3
VI.	Emetine hydrochloride 1 gr. (65 mgm.) s.c. for twelve days and chiniofon 2·5 per cent. retention enemata for the last ten of the twelve days.	50	0

If cysts only are being passed the injections of emetine hydrochloride should be omitted and a start be made with emetine bismuth iodide and chiniofon, followed by diodoquin as for the acute patient. An alternative course has been recommended by Page (1946). Treatment extends over twenty days. Each day three tablets of diodoquin are given three times a day for the twenty days. On the first and second days, 1 gr. of emetine is given intramuscularly. On the third to the fourteenth days (that is for twelve days) 3 gr. of emetine bismuth iodide are given each night and to finish up, on the fifteenth to the twentieth days (for six days), 4 gr. of carbarsone are given daily. The comparative effects of six different courses of treatment have been investigated by Rail (1947), as shown in the table opposite.

For intractable cases which have resisted the above course preliminary treatment with penicillin and sulphasuxidine is advisable. Penicillin should be given in an initial dose of 100,000 units intramuscularly followed by 33,000 units every three hours till 2 million units have been given. Sulphasuxidine is given concurrently in doses of 5 gm. every four hours till 80 gm. has been given. If necessary, sulphaguanidine or sulphadiazine in appropriate doses may replace sulphasuxidine. When there is bacteriological evidence that the amœbic infection is associated with a bacillary dysentery, sulphasuxidine should be given at an early stage.

If, as happens very rarely, a chronic case does not respond to this course, an interval of at least a fortnight should be allowed to elapse before it is repeated, any onset of acute dysenteric symptoms being controlled by hypodermic injections of emetine hydrochloride.

Patients should be given vitamin preparations during treatment and in convalescence despite the fact that the *rôle* of vitamins in amœbiasis in rats is uncertain ; milk may be given from the first, and should be followed by a low residue diet of high caloric value without roughage. Anthimus (*fl.* fifth to sixth century A.D.), personal physician to King Theodoric, in his "De observatione ciborum," written in the first quarter of the sixth century, was an advocate of goat's milk cooked with bread crumbs in an earthen pot. Quince jelly was also recommended : it was prescribed a thousand years later by French physicians. Elsdon-Dew (1949) regards the maize diet of the African in Durban as responsible for fulminating infections, resistant to treatment. The psychological atmosphere should be such as to convince the patient that he will shortly recover.

In amœbic hepatitis and in liver abscess vegetative forms of *Entamœba histolytica* are alone found in the liver. Injections of 0·06 gm. (1 gr.) of emetine hydrochloride for ten to twelve days are usually sufficient to cure an amœbic hepatitis, but the use of emetine injections, merely because the patient suffers from a slightly enlarged and tender liver, cannot be sufficiently condemned. Injections of emetine hydrochloride as a therapeutic test in patients with chronic malaria, infective hepatitis, and liver carcinoma have sometimes led to disaster. If, in a proved case of amœbiasis affecting the liver, symptoms have not abated as a result of emetine hydrochloride injections, an attempt should be made to remove pus by needling. The injection of emetine hydrocloride into a liver abscess does not seem to have any advantage over an ordinary hypodermic injection, but if the abscess is secondarily infected with bacteria penicillin injections should be given. Noth and Hirshfeld (1944), in an amœbic abscess secondarily infected with a Group G streptococcus, successfully introduced penicillin to a total of 830,000 units into the abscess cavity by means of a ureteric catheter.

Tanca Marengõ (1947) believes that in adults with amœbic hepatitis intravenous injections of emetine are preferable to any other method of administration ; 4 mgm. of emetine hydrochloride and 60 mgm. of thiamine are injected intravenously for eight to ten days, followed by the same doses intramuscularly for seven days. Treatment must cease if tachycardia or asthenia develops. It is generally agreed that in all cases of amœbic hepatitis a gut-sterilising course of therapy should follow the relief of the hepatitis. Some chronic cases of amœbic dysentery are then quite easily cured.

Brief reference has already been made to the use of chloroquine in amœbiasis. Chloroquine is known to be concentrated in the liver. Further evidence of the value of chloroquine in amœbic hepatitis was brought forward by Murgatroyd and Kent (1948) and Manson-Bahr (1949). A priming dose of 0·75 gm. is followed by 0·3 gm. or 0·5 gm. daily, and relief of symptoms and fall of temperature occur rapidly. The symptoms may return after one or more courses if the sterile pus is not evacuated surgically.

Amœbic abscesses may occasionally occur in other organs than the liver, but emetine as a rule is successful in treating even an abscess of the brain (Turner, 1948).

It may be noted that infections due to *Isospora hominis* are unaffected by emetine, diodoquin, chiniofon and carbarsone : sometimes spontaneous cure occurs (Barksdale and Routh, 1948).

Criteria of Cure. As amœbic dysentery is notoriously liable to relapse, stools must be examined microscopically during the course of treatment and after the termination of treatment. The question of stool examination is a somewhat difficult one, for cysts from ulcers high up in the colon are likely to be mixed throughout the fæces, whereas cysts from ulcers lower down the bowel are more likely to be found in the outer layer. Smith (1946) has calculated that in persons passing 1,000, 10,000, and 100,000 cysts per day the number of stools to be examined to obtain a 50 per cent. probability of observing one cyst would be 220, 22, and two or three respectively. In practice, careful microscopic examination of stools on six consecutive days at an interval of at least fourteen days after the termination of treatment is usually sufficient to detect the passage of cysts. Sigmoidoscopic examination for test of cure must also be deferred until the same time. If any suspicious ulcer should still persist it should be scraped with a Volkmann's spoon, lubricated with mucilage, as oil droplets make scrapings useless for microscopical examination. In some cases where ulceration may be out of reach of the sigmoidoscope an X-ray examination with a double contrast barium-air enema is of value. In conclusion it may be of importance to summarise the possible causes of failure in the chemotherapeutic treatment of amœbiasis :—

(1) Too rapid excretion of emetine.

(2) Failure of emetine to reach the amœbæ owing to thickening and fibrosis of the intestinal wall as a result of ulcerative colitis (Wenyon, 1947).

(3) Failure of emetine bismuth iodide tablets to disintegrate in the intestine.

If treatment of symptomatic carriers is to be undertaken, either carbarsone, chiniofon or vioform may be used. Arnett (1947) gave one capsule of 0·25 gm. of carbarsone night and morning for ten days to thirteen carriers ; in twelve the parasites disappeared in an average of 1·9 days, in the thirteenth the cysts of *E. histolytica* disappeared only after six weeks, when a second course of carbarsone had been given. Vioform in doses of 0·25 gm. three times daily for a week caused the disappearance of cysts in an average of 1·7 days. Chiniofon was given in doses of 0·25 gm. three times daily for one day, twice daily for four days and three times daily for two days ; of twelve patients thus treated eleven showed loss of cysts in an average of 2·9 days, while the twelfth patient required a second course to eliminate the parasite. In carriers of *E. coli*, *Endolimax nana*, *Dientamœba fragilis*, *Iodamœba*, *Giardia* and *Chilomastix*, administration of carbarsone, vioform and chiniofon causes only a temporary disappearance of cysts.

(4) Physical or biological factors in the gut, such as the *p*H in the lumen of the gut, the nature of the intestinal flora and bacterial invasion of the intestinal wall. In intestinal amœbiasis the *p*H of the gut may shift to the acid side of neutrality (Knowles *et al.*, 1923). Nothing is yet known of the effects of changes in the redox potential in the intestine. It has, however, been shown that in cultures *E. histolytica* leads an anaerobic existence, thriving best at a redox potential of about 0·4 V : greater reduction than this leads to encystment, greater oxidation causes degenerative changes in the amœbæ (Hopkins and Warner, 1946).

(5) Incorrect administration of retention enemata.

(6) Acquired resistance to emetine, of which there is no definite proof.

(7) Failure of collaboration on the part of the patient. Apart from conscious failure to retain the chiniofon enemata certain patients exhibit an unconscious desire to perpetuate their intestinal infection. In the late war, many patients who contracted amœbic dysentery in the tropics improved only after they had reached England. It is now realised that just as mucous colitis is a psychosomatic disease so, in chronic amœbic dysentery, there is also possibly a strong psychic element.

The question of whether symptomless carriers should be treated requires further investigation. The answer depends on whether the passage of cysts in the fæces is regarded as evidence that lesions are present in the bowel. Faust (1941), Craig (1944), and Albright and Gordon (1947) advocate the treatment of all those excreting cysts on the grounds that lesions in the bowel must be present. On the other hand, there is some evidence to show that *E. histolytica* may live in the bowel as a commensal. In addition there are difficulties in treating every carrier in a country where the carrier rate is some 10 per cent. of the entire population and where the chances of reinfection remain constant.

The Action of Drugs on *Entamoeba histolytica*

Very little is yet known of how drugs act on *Entamœba histolytica.* It seems probable that emetine acts by interfering with the multiplication of the trophozoites (Dobell and Laidlaw, 1926), but inability to multiply is merely a sign of some biochemical derangement of the amœba's metabolism. James (1913) noted that emetine caused degenerative changes in the nuclei of the amœbæ and reticulation of the cytoplasm. The arsenicals such as carbarsone presumably combine with sulphhydryl groups in the enzyme systems of the amœbæ, while the antibiotic subtilin has been found by Anderson *et al.* (1946) to cause rupture of the cell membrane. Somewhat similar changes are described by Stewart (1949). The order of appearance of degenerative changes is loss of motility, vacuolation, coarse granulation, central retraction of cytoplasm, loss of nuclear differentiation and, finally, disintegration. Such changes are not specific for emetine and can be found in cultures of *E. histolytica* which are dying in the absence of any drug. Emetine does not have an immediate toxic effect but it has an amœbostatic effect in a dilution of 10^{-3} and some trace of this action even in a dilution of 10^{-7}. Binucleate or dividing cells are absent after four hours' exposure to emetine, and after forty-eight hours' exposure amœbæ have all disappeared from a culture. Emetine does not produce mitotic arrest at a particular stage, nor is its action antagonised by a —SH donor such as cysteine. What exactly is the mode of action of the halogenated oxyquinolines is still unknown. Penicillin appears to act indirectly on the amœbæ by altering their food supply, and the same is true of the synergistic action on amœbæ *in vitro* shown, according to Seneca *et al.* (1949), by a combination of penicillin and streptomycin. It is claimed that aureomycin also has a beneficial action on amœbic dysentery: this again is probably due to interference with the bacterial food supply (McVay, *et al.*, 1949).

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CHAPTER V

THE CHEMOTHERAPY OF BABESIASIS AND OTHER PROTOZOAL INFECTIONS

BABESIASIS

SINCE Nuttall and Hadwen (1909 a, b and c) and Nuttall (1909) first demonstrated that trypan blue, the sodium salt of ditolyl disazo-bis-8-amino-1-naphthol-3 : 6-disulphonic acid, is effective in canine babesiasis, many attempts have been made to find more satisfactory remedies for babesial infections. Although trypan blue is curative in a high proportion of cases, its use is attended by certain very definite disadvantages : if injected subcutaneously if often gives rise to large sterile abscesses which may cause extensive sloughing of the skin and, on healing, unsightly scars. This difficulty may be overcome, it is true, by injecting the drug intravenously, but in small dogs infected with *Babesia canis* the veins are often so collapsed that intravenous injection becomes an impossibility ; in addition, should extravasation of the drug occur, sloughing may take place around the vein. Furthermore, the drug is occasionally toxic to the heart and may cause sudden collapse some fourteen days after injection, even though the parasites have been eliminated from the blood stream.

Trypan blue has been used also in other forms of babesiasis. In cattle infected with *B. bigemina* 1 gm. per 100 kgm. is probably too large a dose as, apart from staining the tissues green for many months, fever, muscular tremor, difficulty in breathing, and acute œdema of the lungs may occur, especially in animals with a heavy infection (Donatien and Lestoquard, 1927 ; Sergent *et al.*, 1945). Doses of 0·05 to 0·1 gm. per 100 kgm. will usually cause the temperature of cattle to fall within twenty-four hours. *Babesia caballi* in horses, and *B. motasi*, are affected by trypan blue, but *B. berbera* is hardly susceptible, and the same is true of *Babesiella major*. Trypan blue is useless also in infections due to *Theileria dispar* and *Anaplasma marginale.*

Pyrrol blue is less toxic but less active than trypan blue; trypan red is quite ineffective against *B. canis* (Meyer, 1912).

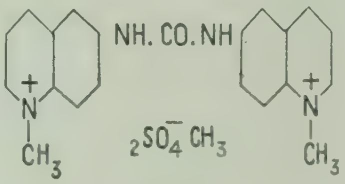
In 1929 Stephan and Esquibel showed that euflavine (trypaflavine) had a curative action in babesiasis. Euflavine is a mixture of 2 : 8-diamino-10-methylacridinium chloride and diaminoacridine monohydrochloride. Acriflavine (gonacrine) itself also has an action on babesiasis. For cattle infected with *B. bigemina*, intravenous injections of from 100 to 200 ml. of 1 per cent. solution cause a fall in temperature in twenty-four hours, but parasites take about a week to disappear from the peripheral blood stream (Sergent *et al.*, 1945). Rampon (1933, 1941) found that in cattle suffering from *B. berbera* some samples of acriflavine were active but others appeared to have no action. For heavy infections four doses of 0·5 gm. at twenty-four hours' interval were sufficient. If the infestation is very heavy fatal reactions may occur. Both trypan blue and acriflavine have been used prophylactically in Russia in horses exposed to infection with *B. caballi*: Greve (1943) recommends injections both in autumn and spring. In cattle exposed to infection with *B. argentina*, 0·5 gm. of acriflavine in a 5 per cent. solution has been used prophylactically with success (Bouhanna, 1942). Acriflavine, euflavine, and trypan blue have the disadvantage that they all stain the tissues, the colour remaining for considerable periods. Acriflavine preparations are useless in infections due to *Babesiella major*, *Theileria dispar* and *Anaplasma marginale*.

Certain silver preparations were found by Yakimoff (1927) to be effective in Russia in the treatment of infections due to *B. bovis*. The most satisfactory preparations were ichthargan, with a 7.1 per cent mortality, arrhena with a 9·1 per cent. mortality, and protargol with a 13 per cent. mortality.

Ichthargan has been found by Sergent *et al.* (1931 and 1945), and Donatien and Lestoquard (1932) to have a slight action in cattle infected with *B. bigemina*. In infections due to *B. berbera*, ichthargan may be given intravenously as a 1 per cent. solution: 1·5 gm. for animals of 200 to 400 kgm. of body weight is satisfactory, though two or three injections may be necessary to remove the parasites from the blood. *Babesiella major* is also susceptible to the action of intravenous ichthargan.

In infections in dogs due to *B. gibsoni*, Kapur (1943), in India, obtained good results with the intravenous injection of neoarsphenamine in a dose of 0·01 gm. per lb.; ten of eleven dogs were cured, while with sulpharsphenamine only five of eleven dogs were cured. Tryparsamide was useless.

A further advance in the chemotherapy of babesiasis was made by Kikuth (1935) with the introduction of acaprin (akiron R, pirevan, piroplasmin, zothelone), *sym*-di-1-methylquinolinium-6-urea methylsulphate.



Acaprin

Acaprin is said to be some eighty times more active than euflavine, and its chemotherapeutic index is eight times as great. *In vitro* a dilution of acaprin in blood, 1 in 10,000, killed *B. bigemina* in twenty-four hours at 20° C. (Battelli, 1942). Acaprin is usually given subcutaneously. In calves infected with *B. bigemina* doses of 5 ml. have some action, but in infections due to *B. berbera* 4 to 12 ml. of a 5 per cent. solution is required (Sergent *et al.*, 1945). Tchernomoretz (1943) did not find any effect from doses up to 2·8 mgm. per kgm. of body weight. Steyn (1942) reported failure to cure *B. bigemina* infection in cows during lactation. In horses in Russia infected with *B. caballi* acaprin was found to be satisfactory in early but useless in long-standing infections. Relapses were common and the mortality reached 30 per cent.; a second course was given after an interval of three to four days (Greve, 1943). In dogs, the usual dose recommended is 0·25 mgm. per kgm. of body weight given subcutaneously, but Carmichael (1935) gave the drug intravenously in doses of 0·02 ml. of a 5 per cent. solution. With both methods the margin of safety is very small and fatal results sometimes occur: according to Carmichael relapses are more common and poisoning more frequent when the drug is given subcutaneously than intravenously. Kikuth and Mudrow (1939) reported that parasitic sterilisation in early cases was possible with doses of 0·25 mgm. per kgm. intravenously, 0·5 mgm. per kgm. intramuscularly and 20 mgm. per kgm. per os ; in long-standing cases parasitic sterilisation was impossible. Of 2,935 sheep infected with *B. ovis* in Iran in 1937, 2,846 were successfully cured (Endrejat, 1938).

Acaprin was found useless by Yakimov *et al.* (1940) in cattle infected with *B. bovis*. The action of acaprin was not increased by the addition of euflavine or urotropin. Bismuth preparations were more active.

The relationship of chemical structure to chemotherapeutic activity in this group of compounds has been studied by Schönhöfer and Henecka (1942) : thus the urea linkage is not essential for action on piroplasms, while azoxy- and azoquinolines are active provided the linkages are at six or seven in the benzene ring : a sulphonamide linkage did not increase the activity.

While acaprin is a definite advance over trypan blue, its very small margin of safety is a serious disadvantage. It appears to be highly dangerous in old horses and in those with cardiac lesions (Guilhon and Vattaire, 1942). *Babesiella major* infections have occasionally been cured by intramuscular injections of 3 ml. of a 5 per cent. solution of acaprin (Sergent *et al.*, 1945). *Babesia trautmanni* which infects pigs is controlled by acaprin, according to Cerruti (1939) and Pavlov and Paschev (1946). The usual dose is 0·5 to 1 ml. of a 5 per cent. solution or 5 to 10 ml. of a 0·5 per cent. solution : if cardiasol is given before the acaprin the danger of toxic reactions is decreased. *Theileria parva* is unaffected (Kikuth, 1938) by subcutaneous injection of the drug. Lourie and Yorke (1939) found that with 0·25 mgm. per kgm. only one of five dogs was cured, and with higher doses, from 1 mgm. upwards, some animals were invariably poisoned. It is to be emphasised that the cure referred to is a complete sterilisation of the infection. In most countries where tick fever (babesia infection) of dogs occurs the disease is enzootic and animals are constantly exposed to reinfection during the rainy season. In these circumstances there is a disadvantage attaching to treatment that will effect a complete cure and thus render the animal open to reinfection. It is essential, however, to free the peripheral circulation from parasites for at least a fortnight, for if a clinical relapse occurs within this period, as is sometimes the case with dogs treated with acaprin, the relapse is usually severe and just as liable to end fatally as the original attack.

The most recent advance in the treatment of babesiasis has been made by Lourie and Yorke (1939), who have shown that a single injection of 5 mgm. per kgm., or two smaller injections of 2·5 mgm. per kgm. of body weight of stilbamidine, propamidine or diamidinodiphenyl ether is sufficient to destroy the parasites. One or two large doses were preferable to repeated smaller doses, owing to the ease with which drug fastness develops. The most active compound was 4 : 4′-diamidino-stilbene, with which three out of five puppies were cured by a single dose of 2·5 mgm. per kgm. of body weight : as 5 mgm. per kgm. appeared to be the maximum tolerated dose, and even that was occasionally fatal, the margin of safety is not great : with propamidine, on the other hand, the margin of safety is greater. Daubney and Hudson (1941), in Kenya, treated sixteen clinical cases of tick-fever in dogs with stilbamidine, the dose in all except one being 1·5 mgm. per kgm. given subcutaneously as a 1 or 1·5 per cent. solution. Of the fifteen animals given 1·5 mgm. per kgm., thirteen made rapid clinical recoveries and in none was any relapse recorded : on the day following treatment parasites could still be seen in blood smears stained with Giemsa, but either they had failed altogether to take the stain or had done so only slightly. After forty-eight hours the blood had become free from parasites and from then onwards it remained free. Two animals died after the injection, but in both the disease was very far advanced. The last animal in the series was given the drug at the rate of 0·9 mgm. per kgm. : though the temperature had fallen to normal and parasites had disappeared from the blood stream within forty-eight hours they reappeared in four days, and in view of the possibility of producing a drug-fast strain the dog was given acaprin and apparently cured.

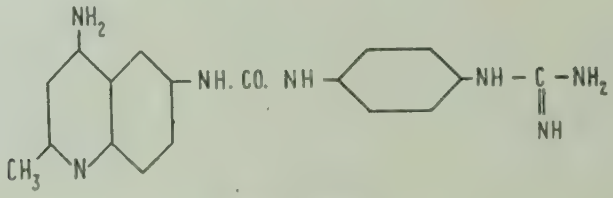
Toxic reactions were seen in all animals receiving stilbamidine. Within a few minutes of receiving the injection they became hyperæsthetic ; they were restless and kept repeatedly rising and walking about : in more than half of them there was transient swelling of the face, and especially the lips, and in most cases the animals showed a wish to scratch the swollen parts. Two dogs had difficulty in breathing. After a period of hyperæsthesia lasting for about twenty minutes, during which time some dogs tried to vomit, the animals usually settled down to sleep comfortable for several hours. On the next day they appeared brighter than before treatment and from then on recovery was progressive.

In Uganda, Carmichael and T-W-Fiennes (1941) treated 116 dogs by propamidine with either 5 mgm. per kgm. of body weight in one dose or 2·5 mgm. per kgm. on two successive days. Subcutaneous, intravenous and intramuscular routes all proved satisfactory, but the last is to be preferred, since œdematous swelling sometimes persists for a month to six weeks when the subcutaneous route is used. Among 116 dogs treated four died while only ten relapsed, and these all responded to a second course of treatment. For dogs propamidine is much less toxic than stilbamidine : there is also the advantage that it keeps indefinitely as a 1 per cent. solution, ready for immediate use. Carmichael (1944) treated 140 dogs with phenamidine, diamidino diphenyl ether, 10 mgm. per kgm. of body weight subcutaneously. Only four dogs relapsed and these were cured after a second course.

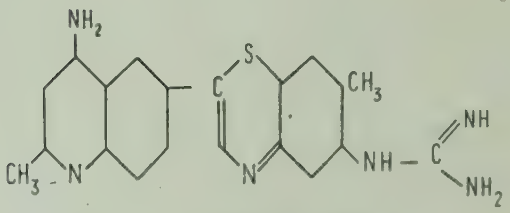
In Palestine, Adler and Tchernomoretz (1940) used stilbamidine in doses of from 2 to 4 mgm. per kgm. in the treatment of infections due to *Babesia ovis* in goats and *Babesia bigemina* in calves. The action on *B. bigemina* is very rapid, and is in evidence one hour after intravenous infection. The nucleus is often extruded, but some parasites retain their nuclei and are able to multiply without regaining their normal morphology. Thus complete eradication of infection is not often produced, but this is an advantage in endemic areas because acquired immunity against *B. bigemina* in non-splenectomised cattle depends on premunition or the maintenance of a residual infection.

The more soluble phenamidine isethionate was used against *B. bigemina* infections in Northern Ireland with considerable success by Pierse (1943), and in Uganda, Randall and Laws (1947) have used the same compound in twenty-four Zebu-Ankole cross-bred cattle. No toxic results were reported when the drug was given in doses up to 22·5 mgm. per kgm. of body weight, but such may occur at doses above this level. When given at a dose of 15 mgm./kgm. all the infections were cured, complete eradication resulting in at least two instances. Phenamidine isethionate had no action on *Theileria mutans* or *Anaplasma marginale.*

During the war two compounds were prepared as a result of modifications in the aminoquinaldine molecule (Surfen), Hoecht 10518 and 10798. Although giving good results in laboratory animals they were found to be too toxic when tested in horses and cattle (Schönhöfer and Henecka, 1942).



Hoeht 10518



Hoecht 10798

Daubney and Hudson (1941) reported the successful treatment of two horses suffering from infections due to *B. caballi* with stilbamidine. Both horses, one a foal, were treated with 1·5 mgm. per kgm. of body weight, but alarming symptoms were produced in both animals. In the foal the muzzle and tongue became swollen and the tongue protruded from the mouth; these symptoms disappeared in a few hours, and in two days the temperature fell from 104° F. to 101·2° F. In an adult thoroughbred symptoms persisted for forty-eight hours. Restlessness and excitement, neighing, profuse sweating, kicking, increased respiration, a jugular pulse, itching, and abdominal discomfort were experienced.

Carmichael (1944) treated a horse infected with *B. caballi* with 3 gm. of phenamidine, the horse weighing approximately 10,000 lb.: parasites disappeared and no toxic symptoms were seen. In *B. berbera* infections of cattle stilbamidine in doses up to 10 mgm. per

THE EFFECT OF TRYPAN BLUE, EUFLAVINE, ACAPRIN AND AROMATIC DIAMIDINES ON THE BABESIDÆ AND THEILERIDÆ

Species.	Host.	Trypan blue.	Euflavine.	Acaprin.	Stilbamidine.	Propamidine.	Phenamidine.
Babesia canis	Dog	+	+ +	+ +	+ +	+ +	+ +
B. bigemina (Texas fever) . . .	Cattle	+	+	+ +	+ +	—	+ +
B. argentina	,,	(+)	+	O	O	—	—
B. bovis (Red water). . . .	,,	+ +	+ +	+ +	—	—	—
B. divergens	,,	O	—	+	—	—	—
B. berbera	,,	O	+	+ +	—	—	—
B. caballi	Horse	+	+	+ +	+ +	—	+ +
B. equi	,,	O	+	+ +	—	—	—
B. ovis	Sheep	O	+	+	+ +	—	—
B. trautmanni (= *B. suis ?*) . .	Pig	—	—	+ +	—	—	—
Theileria parva	Cattle	O	O	—	—	—	—
T. dispar	,,	O	O	+	—	—	—
T. annulata	,,	O	O	+	O	—	—
T. mutans	,,	O	O	O	—	—	O

— = Untested.
O = No action.
+ = Moderate activity.
+ + = Considerable activity.

kgm. of body weight and pentamidine in similar doses were ineffective (Tchernomoretz, 1943). Stilbamidine in doses up to 10 mgm. per kgm. of body weight had no therapeutic action on *Anaplasma ovis*, *A. marginale* or *Theileria annulata* (Adler and Tchernomoretz, 1940). *A. marginale* is said by Sergent *et al.* (1945) and Gonzalo Sotomayor (1947) to respond to treatment with methylene blue or neoarsphenamine. Although atoxyl with antimosan or stibosan is said to improve infections due to *T. annulata* or *T. dispar*, the combination of arsenic and antimony being superior to one alone (Freund, 1929), the majority of observers have failed to find a satisfactory treatment for these conditions (Sergent *et al.*, 1945). Smith and Howell (1944), in an outbreak of anaplasmosis in horses in Oklahoma, found that the most satisfactory compounds were neoarsphenamine, tryparsamide, sodium cacodylate, cobalt chloride and sodium sulphathiazole. Claims have been made that quinoline diphosphate and proguanil cure the symptoms in as high as 85 per cent. of cases. The animals still remain carriers, however. Proguanil alone is a failure (Splitter, 1949). Although trypan blue, acriflavine, and acaprin have all been shown to have some value as prophylactics if injected into animals at the beginning of the tick season, no observations have yet been made on the prophylactic action of stilbamidine, pentamidine or propamidine.

Antibiotics are not usually regarded as of value in protozoal infections. Marney *et al.* (1946), however, claim to have cured a dog with *B. canis* by injecting 8,000 units of penicillin six times a day for three days, followed by 500 ml. of dog blood intraperitoneally.

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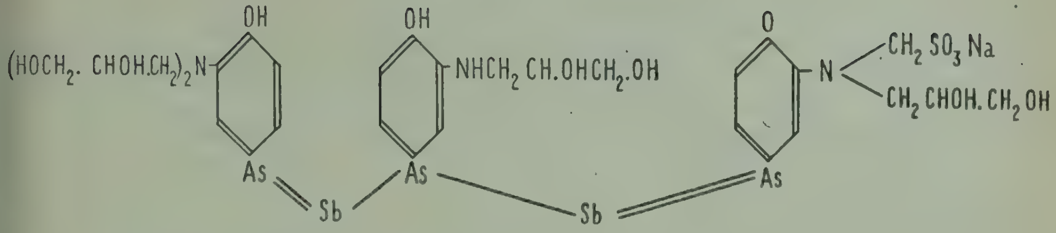
BARTONELLA AND ALLIED INFECTIONS

The most important of this group of parasites is undoubtedly *Bartonella bacilliformis*, the causal agent of Oroya fever.

No really satisfactory chemotherapeutic treatment has yet been found, unless the curative effects of penicillin are fully confirmed. Noguchi (1928), in experimental infections in monkeys, failed to observe any action on the part of either esters and other derivatives of chaulmoogra, or of neutroflavin and proflavin. Prontosil (Carpio, 1939), and the neutral salts of acriflavin, various sulphonamide preparations (Jaramillo, 1939) and quinine (Patiño-Camargo, 1939) all proved ineffective, while Weinman (1943) could not determine that tyrocidine had any *in vitro* action on *Bartonella bacilliformis*.

There is still considerable uncertainty as to the activity of salts of arsenic and antimony. 386B, a product containing 18 per cent. of arsenic and 20 per cent. of antimony (Schmidt and Peter, 1938), was said by Kikuth (1934) to be very effective in *Hæmobartonella muris* infections in rats. Its exact constitution is unknown but the following structure has been suggested :—



The subcutaneous M.L.D. for mice is 0·15 gm. per kgm. Rabbits survive injections of 75 mgm. per kgm. but die in three days after 120 mgm. per kgm. with hæmorrhage of the intestines.

Kikuth (1937 and 1940) has stated that 386B is also specifically effective in Oroya fever in man. His optimism is apparently based on the therapeutic trials of Manrique and de la Rocha (1937, 1938), who found that the drug was tolerated with a highest single dose of 0·3 gm. while the greatest total dosage tolerated was 5·7 gm.; it was usually injected on alternate days, intravenously. Of their cases, fourteen, before treatment, were already anæmic with total red blood cell counts varying from 560,000 to 1,800,000 per c.mm. In this series there were five deaths, but of these none had received more than 1·25 gm. and two had in addition a paratyphoid infection. Of the nine patients who recovered, including the one with a total red cell count of 560,000 per c.mm., six developed verrugas. One patient, who is said to have recovered, received 1·85 gm., after which the blood cultures were still positive. Manrique and de la Rocha concluded that the drug is of considerable value in all stages of the disease. This favourable view, however, has not been shared by others. Jaramillo (1939) found the drug "not satisfactory" in the treatment of Oroya fever. Carpio (1939) treated eleven patients of whom eight subsequently died and three developed verrugas, a result not conspicuously better than that obtained in untreated cases. Weinman (1944) also reported one case where the patient died after receiving 1 gm. in six days; at the autopsy, parasites in large numbers retained their normal appearance and staining affinities.

Other arsenical preparations have been used. In monkeys, neoarsphenamine given in two injections of 0·1 gm. at an interval of two days, or arsphenamine in two injections of 0·05 gm., did not prevent verruga formation but were said by Noguchi (1928) to hasten their regression; in another experiment no regression was seen during a period of six days, following a dose of 0·15 gm. neoarsphenamine (da Cunha and Muniz, 1928). Jaramillo (1939) believed that arsenical preparations are not helpful, while Patiño-Camargo (1939) came to the conclusion that when used in high doses they may actually be harmful.

Merino (1945) treated two patients with penicillin during the initial non-eruptive phase of the illness. Two courses were given to each patient: the first received two courses of 300,000 Oxford units, the second 300,000 and 800,000 units. The degree of red cell parasitism fell and degenerative changes were seen in the parasites. Delgado (1945) also reported similar changes in one patient, and Vila Acuña (1945) noted regression of nodules in another.

In the hands of Aldana (1946), penicillin has been found to have an action on *Bartonella* both *in vivo* and *in vitro*. In cultures of bartonella giant forms are seen at the end of two to three days, and by six days the parasites are dead. In the anæmic phase of Oroya fever penicillin produces a remarkable improvement in twenty-four hours. On the eruptions the effects are less dramatic but the lesions tend to disappear after from 500,000 to 2,000,000 units. The daily dose should be from 100,000 to 200,000 units.

Hæmobartonella muris of rats belongs to the genus *Hæmobartonella*, which has been separated from the monotypic genus *Bartonella* by Tyzzer and Weinman (1939). *Hæmobartonella* produces anæmia but no cutaneous eruption.

A true sterilisation of the latent or declared infection with organic arsenical compounds was obtained by Mayer *et al.* (1927). The anæmia in rats is curable by very small doses of neoarsphenamine, given orally or subcutaneously (1·4 mgm. per kgm. of body weight): with 3 mgm. per rat the disease does not appear after splenectomy. Vassiliadis (1930–1931) for certain sterilisation recommends three injections of 10 mgm. per 100 gm. of body weight with a few days' interval between each.

Neoarsphenamine was found by Mayer *et al.* (1927) to have a chemotherapeutic index of seventy-two, while that of tryparsamide was 4·8. Sodium arsenite and sodium cacodylate, chiniofon, bismuth as "pallicide," methylene blue, trypaflavine, suramin, sodium salicylate, tartar emetic, and antimosan were inactive.

Uhlenhuth (1931) and Uhlenhuth and Seiffert (1933) obtained a slight action with a quinquevalent antimony preparation, stibosan having a chemotherapeutic index of 1 : 8. Compounds of undisclosed composition containing arsenic and antimony, referred to as 283B, 246B, and 386B, had very high chemotherapeutic indices of 1 : 300 to 400, 1 : 400 to 500 and 1 : 3,500. Std. 386 B is fatal in rats in doses of 750 mgm. per kgm. of body weight injected subcutaneously ; 500 mgm. per kgm. is tolerated by all rats, while 0·5 mgm. per kgm. causes the disappearance of *H. muris* for a few days.

Sulphanilamide had no effect (Emery, 1940), and penicillin is also without action (Ubatuba and Vieira, 1944), results confirmed by Archetti (1947). The appearance of drug fastness, has been described by Mayer and Malamos (1936). Some observers have found an improvement in the anæmia associated with *Hæmobartonella muris* following injections of copper and iron : others have failed to note any ameliorative effect from these metals, either alone or in combination. There is, however, agreement that neither metal has any direct antiparasiticidal effect.

Hæmobartonella canis infection can be completely eradicated by neoarsphenamine in a dose of 15 mgm. per kgm. of body weight. Smaller doses cause a temporary disappearance of the parasites. In the white mouse the specific *Hæmobartonella* is more difficult to eradicate than in the rat, neoarsphenamine, according to Kikuth (1932), having a chemotherapeutic index of only 1 : 5. Domagk and Kikuth (1933) reported successful treatment with compounds of arsenic and antimony. Penicillin failed with *H. canis* (Davis, 1949).

Whether *Eperythrozoon* should be classed with bacteria or with protozoa is still a subject of discussion (Weinman, 1944). In their response to chemotherapeutic agents these organisms resemble more closely the spirochætes than the true bacteria. *E. coccoides*, which is found in splenectomised mice, is readily influenced by the same drugs as are effective against the *Hæmobartonellæ*. The sterilising dose of neoarsphenamine is 2·5 mgm. for a mouse weighing 15 to 20 gm. ; sulpharsphenamine causes only a temporary disappearance, followed by a recurrence in fifteen to twenty days' time (Vassiliadis, 1930–1931). The initial dose, followed a day later by one half the quantity (1·25 mgm.), eliminates the infection (Tyzzer, 1941). A dose of 20 mgm. per 20 gm. of body weight of tryparsamide causes a temporary disappearance of the parasite from the blood stream for a period of four or five days, but Vassiliadis (1930–1931) could not determine any activity on the part of stibosan, tartar emetic, quinine sulphate or suramin.

E. ovis was first described in sheep in South Africa. Neoarsphenamine in the maximum tolerated dose of 45 mgm. per kgm. of body weight causes a temporary disappearance of parasites from the peripheral blood stream for as long as twenty-nine days. Sdt. 386 B apparently produces complete sterilisation ; doses of 10, 20 and 30 mgm. per kgm. were injected into two sheep ; parasites disappeared from the peripheral blood stream within one hour and remained absent for 110 days, when the sheep were reinoculated : five of the six then reacted like previously uninfected animals (Neitz, 1937); doses of 5 mgm. per kgm. led to premunition. Neoarsphenamine in a heifer, infected with bovine eperythrozoonosis due to *E. wenyoni*, caused disappearance of the parasites from the blood when injected in a dose of 2·25 gm. for a live weight of 180 kgm. The blood remained parasite-free for 112 days, the period of observation, but it is not certain that complete sterilisation was attained (Nietz, 1940). Acaprin also caused rapid disappearance of parasites from the blood, but relapses occurred after short intervals (Delpy and Rafyi, 1938).

Aldana *et al.* (1948) described a case of verruga where the organisms became resistant to streptomycin but remained sensitive to penicillin.

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TOXOPLASMOSIS

A considerable number of chemotherapeutic agents have been tested against toxoplasma both *in vitro* and in experimental animals. Warren and Sabin (1942) found that mepacrine in a dilution of 1 in 50,000 inactivated toxoplasma after less than three hours' incubation. Neoarsphenamine, tryparsamide, mapharsen, trypaflavine, rivanol lactate, potassium antimonyl tartrate, optochin and quinine hydrochloride were also effective *in vitro* after more prolonged periods of incubation. However, when these compounds were tested in experimental animals they were without action. Sulphapyridine and sulphathiazole, although without action *in vitro*, were found to have an inhibitory action on infected mice, for when injected with 1,000 to 10,000 M.L.D. of toxoplasms the mice remained healthy as long as they were given these drugs by mouth: when drug therapy was stopped the animals eventually died of toxoplasma infection. If large doses of sulphathiazole and sulphapyridine were given parenterally to mice the infection, according to Warren and Sabin, was apparently cured. Further investigations by Weinman and Berne (1944) showed that although the sulphonamide drugs, particularly sulphapyridine and sulphadiazine, might be successfully used to cure the acute stage of the disease, yet the infection was not eradicated and the animals remained carriers. The infection could be transmitted to uninfected mice by inoculation of a brain suspension from the treated animals, while histological examination of the brains of mice which had received sulphonamides disclosed toxoplasmas in the tissue. Sulphathiazole was less efficient than sulphapyridine or sulphadiazine, and treatment had to be begun within five days of infection. There is thus a very close analogy with the effect of sulphonamides on mice infected with toxoplasms and with the virus of lymphogranuloma venereum.

Robinson (1947) successfully treated a patient with toxoplasmic meningoencephalitis with sulphathiazole. A girl, aged nine years, was given a primary dose of 1·5 gm. per os followed every four hours by 0·75 gm. for twelve days; in addition 0·015 gm. emetine hydrochloride was given intramuscularly daily. The symptoms subsided after nine days and the patient was discharged.

Later she complained of severe headache ; the temperature rose to 102·6° F., and Babinski's sign became positive on both sides. Sulphathiazole and emetine were resumed for six days ; after two days' treatment the temperature became normal and all neurological symptoms disappeared. Recovery was complete and no relapse occurred.

Biocca and Nobrega (1945) believe that sulphadiazine and certain sulphones in oil are more effective than sulphathiazole.

Kugelmass (1948) cured a four-year-old boy with sulphapyridine, which was given in doses sufficient to maintain a blood concentration of 5 to 10 mgm. per 100 ml. : folic acid was given for the anæmia and prostigmin for the spasticity. Sulphamylon hydrochloride (4-aminomethyl benzene sulphonamide hydrochloride) was ineffective in mice (Cross and Anigstein, 1948).

Augustine, Weinman and McAllister (1944) have reported that penicillin is without effect in toxoplasma infections. Cross and Anigstein (1948) found streptomycin alone and in combination with *p*-aminobenzoic acid ineffective. Chloroquine and toluidine blue were likewise useless.

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BALANTIDIOSIS

Although infection with *Balantidium coli*, a parasite of pigs, is by no means common, there is little doubt that it has pathogenic properties in man and that it can produce dysenteric symptoms.

The long list of medicaments which has been recommended for this condition speaks for the lack of a really specific therapy.

Oil of chenopodium was given per rectum to twelve patients by Cort (1928), but Serra (1931) reported the death of a child from chenopodium poisoning as a result of this treatment, and Young (1939) found it ineffective in four patients. Owing to the similarity of the lesions of balantidial dysentery to those produced by *Entamœba histolytica*, amœbicidal drugs have been used. Atiles (1943) believed that he had successfully eliminated the parasite in two children by means of acetarsol. Young (1939) employed carbarsone, the dose varying from 0·1 to 0·5 gm. daily and the total dosage from 2·2 gm. in fourteen days to 5 gm. in ten days. Trophozoites appeared dead in from six to fourteen days. Meleney (1939) found that one patient relapsed after oral carbarsone; when the administration of carbarsone by mouth was combined with retention enemata of 2 gm. of carbarsone dissolved in 200 ml. of a 1 per cent. solution of sodium bicarbonate, the patient was cured. Anderson *et al.* (1948) suggested the use of thioarsenites.

Young and Burrows (1943), in reporting the effect of carbarsone treatment on balantidiosis, emphasise the necessity of following up the patients for long periods. While Tsuchiya and Kenamore (1945) were satisfied with the use of carbarsone, DeLanney and Beahm (1943) reported that treatment by carbarsone followed by oil of chenopodium gave only temporary relief. Diodoquin, however, in doses of 2·5 gm. for twenty days, freed the patient from parasites for at least eighteen months. Hummel (1940) had previously reported on the disappearance of lesions and parasites from the stools of a patient given 2·1 gm. of diodoquin daily for ten days; as, however, no further observations were made, the permanency of the cure is uncertain.

Intramuscular injections of biniodide of mercury are said to have cured eight of nine cases (Shun-Shin, 1947). Adults received 32 mgm., children were given one or two injections of 2·7 to 6·5 mgm., according to age. The biniodide of mercury is *in vitro* ten times more lethal for *Balantidium* than mercury perchloride. Lorie (1948) still believes mepacrine to be of value.

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BLACKHEAD OF TURKEYS

Another protozoal disease of considerable veterinary importance is blackhead of turkeys due to *Histomonas meleagridis.* The most impressive results have been obtained by DeVolt and Holst (1948), who gave 1 per cent. of iodochloroxyquinoline (vioform) in the food. The effects of giving the drug for forty-eight hours before infection on the survival of young turkeys are shown in the table on p. 254. Vioform also exerts a preventive action against artificially induced blackhead of turkeys. A field experiment in an infected flock showed that of fifty-one turkeys receiving 1 per cent. of vioform in the mash only ten died, whereas of fifty-three control untreated birds twenty-four died from the disease in the same period.

Sulphathiazole, 0·5 and 1 per cent., and sulphaguanidine in the same amounts were useless. The preventive action of vioform was confirmed by Morehouse (1948); chiniofon and 5-chloro-6-iodo-8-hydroxyquinoline, at doses of 0·5 and 1 per cent. in the food, were ineffective when given for fourteen days, beginning three days before infection.

THE PREVENTION OF BLACKHEAD IN TURKEYS BY VIOFORM (IODOCHLOROXYQUINOLINE).

Group.	Number of poults inoculated.	Number of histomonads inoculated.	Number of inoculations.	Interval between first inoculation and symptoms in days.	Average length of life.	Number dead.	Number surviving.	Survivals, rate per cent.
1	Medicated 10	$5{\cdot}5 \times 10^5$	4	11	19·2	5	5	50
	Control 11		4	4	10·2	11	0	0
2	Medicated 20	8×10^5	4	—	—	—	20	100
	Control 8		4	8	12	7	1	12·5
3	Medicated 9	$3{\cdot}5 \times 10^5$	4	15	18	1	8	88·8
	Control 9		4	5	11·7	9	0	0
	Totals :							
	Medicated 39	—	—	—	—	6	31	84·7
	Control 28				—	27	1	3·6

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COCCIDIOSIS

Coccidiosis attacks the majority of domesticated animals and birds, but is of special importance in young chickens and rabbits. Lambs, calves, pigs, dogs, and cats are also seriously affected.

Coccidiosis in Chickens

Infection with various species of *Eimeria* is a cause of considerable mortality among very young chickens. Before the war it was known that flowers of sulphur, if given in large doses before infection and before symptoms had appeared, improved the condition of chickens infected with *Eimeria tenella* or *E. necatrix*; it did not, however, eliminate the parasites. When 5 per cent. of sulphur was added to the diet, though the mortality decreased, various toxic effects were seen (Levine, 1941b), the kidneys, especially, being liable to damage.

Various methods have been used in an attempt to increase the action of sulphur while decreasing its toxicity. Thus Goff (1942a and b) mixed with the food 5 per cent. of sulphur and 5 per cent. of finely powdered charcoal. The period for which the mixture is effective against *E. tenella* is five times as long as when sulphur is given alone. Carpenter (1940) employed aqueous colloidal sulphur added to drinking water or given in the mash. In drinking water 1 per cent. of colloidal sulphur protected against from 7,500 to 100,000 mature oocysts of *E. tenella :* in the food, concentrations of from 2 to 7 per cent. of sulphur were non-toxic, but if larger amounts were added fatal nephritis developed.

Harwood and Guthrie (1943) tried the prophylactic effects of triethanolamine hydrochloride when given as 4 to 5 per cent. of the food. Micronised, wettable sulphur (0·5 per cent.) and urea reduced deaths by two-thirds. Herrick *et al.* (1942) used dodecyl thiocyanate, " lorol " thiocyanate, and tetra-ethylthiuram monosulphide. All these had some effect, but a watery emulsion of the last compound with 25 per cent. coconut oil was effective only in doses which approached the toxic.

Since the advent of sulphonamides little has been heard of the use of sulphur, while the same is true of borax, which could be given only for twenty-four hours ; it must cover the period from seventy-two to ninety-six hours after infection (Hardcastle and Foster, 1944). Kay (1947) found that in effective concentrations it retards growth. Morehouse and Mayfield (1946) studied the effects of certain aryl arsonic acids in experimental coccidiosis of chickens. The two best preparations against *E. tenella* were 4-hydroxy-phenylarsonic acid, and 3-nitro-4-hydroxy-phenylarsonic acid. The former had some effect on *E. acervulina*, but effective doses were at a toxic level. If birds survived treatment, however, they developed some immunity to subsequent infection. Morehouse (1946) found that some halogenated arsonic acids such as 2-chlorophenylarsonic acid, 4-chlorophenylarsonic acid, and 4-bromophenylarsonic acid prevented infection if given at the same time as the infecting dose of *E. tenella :* hæmorrhages could be controlled by concentrations of 0·0026 to 0·0066 per cent. in drinking water. Alkyl derivatives of dithiocarbamic acid are highly toxic to various species of *Eimeria in vitro* (Tisdale and Flenner, 1942); they do not appear to have been tested *in vivo*.

Nitrofurazone, 5-nitro-2-furaldehyde semicarbazone, was found by Harwood and Stunz (1949) to have some effect on avian coccidiosis. The median lethal dose for chicks is between 150 and 200 mgm. per kgm. of body weight, but as little as 1 part in 9,000 parts of food is effective if given fifty-six hours after infection. Some effect is noticeable if the drug is not given till sixty-four hours after infection. There is no interference with the development of immunity nor with growth, as the food is freely eaten by hens. There is considerable loss of action if the drug is dissolved in water and placed in iron troughs, as nitrofurazone undergoes some reaction with the iron. Nitrofurazone would seem to be more satisfactory than sulphur or the arsonic acids and as satisfactory as sulphaguanidine. It is probably less satisfactory than sulphamezathine.

A considerable advance was made in the chemotherapy of avian coccidiosis by the discovery that sulphonamides had an action on various species of *Eimeria.*

Levine (1939a) was the first to show that sulphanilamide inhibited the development of the oocysts in five of the species of coccidia parasitic in the fowl. The drug was either mixed with the food in amounts of from 0·1 to 0·5 per cent. of the body weight or given daily in a capsule containing 0·3 gm. to birds approximately 1·5 lb. in weight. When sulphanilamide was given in 0·1 to 0·2 per cent. doses there was an effect on the production and discharge of oocysts of *Eimeria hagani* or *E. præcox*, but no dose could be found which affected *E. tenella* or *E. necatrix*. After cessation of drug treatment the number of oocysts of susceptible species again increased, but never to the same number as in untreated birds. With the advent of sulphapyridine Levine (1939b) found that though there was again no effect on *E. tenella* or *E. necatrix* the action on *E. præcox*, *E. mitis* and *E. hagani* was more pronounced than with sulphanilamide.

Sulphathiazole was effective prophylactically against *E. tenella* when given to the extent of 1 or 2 per cent. in the food before or at the time of infection, but the blood concentration obtained was low (Ripsom and Herrick, 1945).

The next sulphonamide to be tested was sulphaguanidine, the absorption of which from the intestine is much less than in the case of sulphanilamide or sulphapyridine. Fed to chickens at the rate of 0·5 per cent. of the ration, sulphaguanidine was shown by Levine (1941a and 1943) to suppress all species of coccidia except *E. tenella* and *E. necatrix*: if the amount of sulphaguanidine in the food was increased to 1 or 1·5 per cent., then the number of oocysts of these two species was also reduced, although complete eradication was not seen. Sodium sulphanilyl sulphanilate, sodium 2-sulphanilamidobenzoate monohydrate, and sodium disulphanilamide were far less effective than sulphaguanidine (Levine, 1941a). The effects of 0·5 per cent. in the food of sulphanilamide, sulphapyridine, sulphathiazole and sulphaguanidine were compared by Levine (1942): although all species except *E. tenella* and *E. necatrix* were eliminated, medication in either the pre-clinical or clinical stages of the disease was of little value; it was essential to begin drug treatment before the birds were infected.

The effects of sulphaguanidine on *E. acervulina* were studied by Levine (1941a, 1943) and Farr (1949). If given in a concentration of 0·5 per cent. for four days at the time of inoculation sulphaguanidine is primarily coccidiostatic. Within four days of ending therapy birds become sick: if given one to two days after infection there is a four-day delay in oocyst discharge and a moderate reduction in output; birds do not put on weight. If treatment is delayed for three or four days after infection the output of oocysts is reduced and symptoms are prevented. Treatment for four or five days after the onset of clinical symptoms does not eliminate the parasite nor is there evidence of resistance to reinfection if birds are reinoculated eighteen days after the cessation of treatment.

The value of sulphaguanidine as a prophylactic in chickens was further investigated by Farr and Allen (1941) and Allen and Farr (1943). If birds were given 1 or 2 per cent. of sulphaguanidine in their diet before infection no symptoms of disease were noted, but no immunity or premunition developed and the birds were highly susceptible to infection one month later. A dose of 0·5 per cent. of sulphaguanidine for three days, while it suppressed symptoms, gave rise to a considerable degree of immunity to a subsequent exposure to infection and the weight gains of treated birds were as good as uninfected controls. Wehr and Farr (1945) also showed that 0·5 per cent. of sulphaguanidine in the food reduced the number of oocysts of *E. tenella* if treatment began one day before or within two days of experimental infection and was continued for seven days ; 1·2 per cent. of drug in the food begun three days after infection reduced the mortality.

Sulphadiazine, given to the extent of 1 per cent. in the food at the time of infection or within three days of infection, reduced the intensity of infection due to *E. tenella*, but the viability of oocysts already formed in the tissues was not affected (Ripsom and Herrick, 1945). Sulphadiazine, however, was toxic if given to the extent of 2 or 3 per cent. in the diet. Sulphadiazine and sulphadimethylpyrimidine (sulphamezathine) had, however, both been previously examined by Horton-Smith and Taylor (1942), who regarded them as superior to sulphaguanidine, since they prevented infection if their administration was delayed for seventy-two to ninety-six hours after administration of oocysts, whereas sulphaguanidine had to be given at the same time as the oocysts.

Later it was shown that a convenient way to administer sulphamezathine was to substitute a saturated solution of the drug for the drinking water. By this means it was possible entirely to prevent infection by a massive dose of oocysts given seventy-two hours previously, and almost complete protection was obtained after an interval of ninety-six hours. Even if treatment were delayed until deaths began to occur in the untreated chickens it was still possible to save 90 per cent. of birds by this treatment where among the untreated birds only 45 per cent. recovered spontaneously (Horton-Smith and Taylor, 1943). An important point, however, was found to be the provision of a fresh solution daily for if the sulphamezathine solution were allowed to stand in contact with metal drinking vessels, decomposition occurred and the curative effect was decreased (Hawkins and Kline, 1945). Further investigations have amply confirmed the beneficial results obtained with sulphamezathine, provided the blood level is at least 5 to 6 mgm. per cent. by the fifth day of treatment (Hawkins, 1943 ; Swales, 1944). Treatment should be continued for six or seven days from the time when hæmorrhage is first noted ; the mortality is then from 50 to 73 per cent. below that in untreated birds, while over a period of twelve days gains of weight in from 50 to 60 gm. are noted in treated birds as compared with an average gain of 1 gm. in untreated birds. Wehr and Farr (1947) found little or no benefit when treatment was initiated on the fourth, fifth or sixth day after inoculation whether the drug was given in 1 per cent. medicated mash or for two days in 0·25 gm. capsules. Treatment begun at the time of inoculation or within three days thereof materially benefited the birds. Heavily infected birds treated with sulphamezathine show a high degree of immunity to reinfection (Horton-Smith and Taylor, 1945).

No toxic symptoms are noted in birds treated for seven to ten days, but a curious effect resulting from the administration of sulphamezathine was observed by Asplin *et al.* (1946). If young chickens are given a saturated solution of the drug as a substitute for drinking water for two or more weeks the males exhibit precocious development of the comb and wattles and testicular enlargement due to hyperplasia of the seminiferous tubules. This effect, which is apparently common to other sulphapyrimidines, is associated with a prolongation of the clotting time of the blood and with the occurrence of widely distributed petechial hæmorrhages. P'an (1948) was unable to confirm the action of sulphamezathine on the testes. The action of 0·2 per cent. in the food of young rats was to cause testicular atrophy.

Other sulphapyrimidines are also active in killing coccidia : thus Swales (1946a and b) found that sulphamerazine or its sodium salt will check the disease, if given soon after bleeding occurs, in doses of 2 gm. per lb. of feed or 2 gm. of the sodium salt per litre of drinking water for three days : no toxic effects were seen. Farr and Wehr (1945) showed that 1 per cent. of sulphamerazine reduced the mortality if given in the food, but toxic effects were noted and the increase in weight was retarded. If sulphamerazine were given before or within one day of infection, the disease tended to reappear after a latent period of from four to six days : when the drug was given in the food to the extent of only 0·25 per cent. it was equally effective if given within two days of infection. Thorp *et al.* (1946a and b) also used sulphamerazine: 0·2 per cent. sodium sulphamerazine added to the drinking water at the first symptom of cæcal coccidiosis lowered the mortality and in field trials controlled outbreaks of cæcal coccidiosis. This amount of sodium sulphamerazine and 0·25 per cent. in the mash had prophylactic properties and did not appear to affect the production of immunity to the strain of *E. tenella* used.

Somewhat contradictory results have been obtained on the toxicity of sulphamerazine for chickens. Welch *et al.* (1943) thought that daily administration of 0·58 to 0·88 gm. per kgm. to chickens averaging 778 gm. in weight did not cause loss of weight but produced lesions in the sciatic nerve, cord, and kidneys. Mattis *et al.* (1946) observed no injurious effects from feeding 0·5 to 1 per cent. concentrations of sulphamerazine in the food for fourteen days. Farr and Jaquette (1947) found that doses of more than 0·25 per cent. daily retarded growth and caused hæmorrhagic lesions in the spleen. In rats, 1 per cent. of sulphamerazine in the diet caused a reduction in the bone marrow of stab cells, metamyelocytes and neutrophil leucocytes. Nucleated red cells were increased and gross hæmorrhage was found in the subcutaneous tissues and in the retroperitoneal fat (Farr and Jaquette, 1947). Both sulphamerazine and sulphamezathine may have a temporary inhibiting effect on egg-laying (Bankowski, 1947).

Waletzky and Hughes (1946), who tested forty-five different sulphonamide compounds in avian coccidiosis, came to the conclusion that sulphapyrazine was extremely effective, a conclusion at which Horton-Smith and Boyland (1946a) also arrived independently. A solution containing 0·1 per cent. of sulphapyrazine, instead of 0·2 per cent. as in the case of sulphamezathine, was active when substituted for the drinking water and was free from the toxic effects associated with sulphamezathine. Chickens which survived sulphapyrazine treatment had developed a good immunity.

Waletzky and Hughes (1946) believed that 2-sulphanilamido-5-chloropyrimidine and the analogous bromopyrimidine were nearly twice as active as sulphapyrazine and sulphamezathine. On the other hand, Swales (1946a and b) found that while sulphadiazine, the monomethylated compound sulphamethylpyrimidine (sulphamerazine) and the dimethylated sulphadimethylpyrimidine (sulphamezathine) are active, the fully methylated drug, 2-sulphanilamido-4, 5, 6-trimethylpyrimidine and 2-sulphanilamido-4, 5-dimethylpyrimidine are inactive.

The relative therapeutic effectiveness of sulphapyrazine and the sulphapyrimidines in controlling coccidiosis has been shown by Horton-Smith and Boyland (1946b) to depend on the blood concentrations obtained. Effective blood concentrations should be nearer 10 than 5 mgm. per 100 ml.

The value of a number of sulphonamides in the prevention and treatment of infection due to *E. tenella* is shown in the table. There is a certain parallelism between the activity of sulphonamides on this coccidium and on *Plasmodium gallinaceum*, and, just as the effect of sulphonamides on the malarial plasmodia is inhibited by *p*-aminobenzoic acid, so is the action on *E. tenella.*

The cytological changes produced by sulphamezathine on *E. tenella* have been studied by Farr and Wehr (1946 and 1947) and Horton-Smith (1948). When sulphamezathine is added to the food to the extent of 1 per cent., sporozoites and many first-generation schizonts develop normally. Some first-generation schizonts, however, become vacuolated and degenerate into a dark-staining mass surrounded by fragments of cytoplasm. The majority of the large, second-generation schizonts undergo degenerative changes similar to those seen in schizonts of the first degeneration. Merozoite formation is thus greatly reduced and only a few gametocytes are produced : those gametocytes that are produced develop normally to maturity. As Horton-Smith (1948) points out, these effects are very similar to the inhibition of plasmodia by antimalarial drugs. A concentration of 10 mgm. per 100 ml. of blood is necessary to inhibit nuclear division and segmentation into merozoites. Even with 0·2 per cent. in the diet nuclear division and segmentation into merozoites is inhibited. The schizonts of the second generation are shrunken and are from 28μ to 30μ in length instead of about 50μ.

According to Steward (1947), sulphones are of some value in avian coccidiosis : 4 : 4′-diaminodiphenyl sulphone has some activity against the duodenal coccidium *E. acervulina* and to a lesser degree against *E. tenella*: it is more active than 4 : 4′-dichlorodiphenyl sulphone.

THE ACTION OF SULPHONAMIDES ON INFECTIONS DUE TO *Eimeria tenella* IN CHICKENS

Compound.	Action on *Eimeria tenella* infection.	Number of hours' delay from infection to beginning of treatment.	Authority.
Sulphanilamide	No prophylactic effect.	—	Levine (1939a).
Sulphapyridine	No prophylactic effect.	—	Levine (1939b).
Sulphathiazole	1 to 2 per cent. effective in food before or at time of infection.	—	Ripsom and Herrick (1945).
Sulphaguanidine	1 to 1·5 per cent. effective in food before infection. 0·5 to 1 per cent. effective.	—	Levine (1941a).
Sodium sulphanilyl sulphanilate.	No prophylactic effect.	—	Levine (1941a) and Swales (1947).
Sodium 2-sulphanilamido-benzoate.	No prophylactic effect.	—	Levine (1941a).
Sodium disulphanilamide.	No prophylactic effect.	—	Levine (1941a).
Sulphadiazine	1 per cent. in food effective.	72 hours.	Ripsom and Herrick (1945).
Sodium sulphadiazine	0·1 per cent. in drinking water effective.	24 hours.	Horton-Smith and Boyland (1946a, b).
Sodium sulphamerazine	0·2 per cent. in drinking water effective.	72 hours.	Swales (1946).
Sodium sulphamezathine	0·2 per cent. in drinking water excellent effect.	24–72 hours.	Horton-Smith and Boyland (1946). Horton-Smith and Taylor (1943).
Sodium sulphapyrazine	0·1 per cent. in drinking water excellent effect.	24–72 hours.	Horton-Smith and Boyland (1946a and b)
2-Sulphanilamido-5-chloropyrimidine.	0·1 per cent. in food very effective.	—	Waletzky and Hughes (1946).
2-Sulphanilamido-5-bromopyrimidine.	0·1 per cent. in food very effective.	—	Waletzky and Hughes (1946).
2-Sulphanilamido-4, 5, 6-trimethylpyrimidine	Inactive.	—	Swales (1946a, b).
2-Sulphanilamido-4, 5-dimethylpyrimidine.	Inactive.	—	Swales (1946a, b).
Sulphaquinoxaline	0·0125 per cent. in food effective.	—	Peterson (1948).

Sulphaquinoxaline, 2-sulphanilamido-quinoxaline, is said to be effective against *E. tenella* and *E. necatrix* given in the food of chickens at the rate of 0·05 per cent. intermittently and 0·0125 per cent. continuously (Delaplane *et al.*, 1947 ; Grumbles *et al.*, 1948).

Grumbles and Delaplane (1948) believe sulphaquinoxaline to be three or four times as effective as sulphamethazine. All these compounds if given four days or more after infection are unreliable (Seeger, 1947 ; Swales, 1947 ; Peterson, 1948).

Phenothiazine appears to be of no value in avian coccidiosis. Baldwin *et al.* (1941) found that vitamin K reduced the hæmorrhage in chickens infected with *E. tenella.*

Mepacrine is also of value in coccidiosis of fowls due to *E. tenella.* Wilson (1949) carried out a series of experiments in which 0·1 gm. of mepacrine hydrochloride was dissolved in 4 pints (1·89 litres) of water and given to chicks twenty-four hours after infection. The results were as follows :—

Of 40 chickens, 10 days old . .	7	died with coccidiosis
,, ,, control chickens 10 days old .	26	,, ,, ,,
,, ,, chickens 21 days old . .	7	,, ,, ,,
,, ,, control chickens . . .	23	,, ,, ,,

Chickens drank this water for six weeks and showed no loss of appetite : their feathers, however, were a little ruffled and their weight on the average was 3 oz. (45 gm.) below that of normal controls. Three weeks after the end of the experiment the weight of the medicated chickens was equal to that of the normal controls.

No attempt seems to have been made to compare the effects of mepacrine with those of the sulphonamides.

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Coccidiosis in Rabbits

Very few observations have been made on the effect of sulphonamides on the coccidia of rabbits. Joshua (1943), however, brought forward evidence to show that sulphaguanidine was of value. Rabbits could be given either large doses, such as 0·5 gm. per kgm. of body weight for two days or the same total dosage spread over a considerable period. The former method appeared to be the more satisfactory, and two courses could be given without untoward results at an interval of ten days. Apart from constipation for from twenty-four to thirty-six hours there were no toxic results. Gerundo (1948) believes that hepatic coccidiosis can be controlled by succinylsulphathiazole.

Mepacrine was used by Vogelsang *et al.* (1939) and Gallo and Loretto (1940); for rabbits twelve to fifteen days old 0·5 ml. of mepacrine 1 in 1,000 is given intravenously on three successive days, while in rabbits from one to two months old 1 ml. is given intravenously on four successive days. Sometimes eight injections are necessary to destroy all parasites. All rabbits so treated are said to have recovered whereas 90 per cent. of untreated rabbits died from the infection. These results have been confirmed by Brumpt (1942, 1943a and b), who reported a considerable reduction in the mortality of rabbits given mepacrine intravenously, although the infection was not completely eradicated.

Phenothiazine is claimed by Hegnell (1942) to be of value in reducing the mortality in rabbit coccidiosis. For rabbits under three months old the dose, mixed with the food, was 0·25 gm., for those three to six months old 0·5 gm., and for full-grown rabbits, 1 gm. The treatment was repeated after an interval of one or two weeks.

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Coccidiosis in other Species

Bovine coccidiosis is usually due to *Eimeria zürnii* and *E. bovis*: the first causes severe diarrhœa or dysentery, the second only mild abdominal discomfort. Many drugs, including ipecacuanha, have been tried without success. The condition was treated with mepacrine by Perrin (1942) and Edgson (1948), the drug being given either orally or intravenously. By mouth, 0·8 gm. was given morning and evening dissolved in 1 litre of water, while intravenously 1·5 gm. was given dissolved in 40 ml. of water. Of thirty-five severely infected cattle thus treated only one died. Brumpt (1943a and b) gave 1 gm. per 100 kgm. of body weight for two days by mouth, and for intravenous injection employed 1 gm. per 100 kgm. body weight dissolved in 20 ml. of sterile water.

Sulphanilamide is said by McPeek and Armstrong (1941) to have destroyed all oocysts in five days in infected cattle when given daily by mouth at the rate of 1 gm. per 20 lb. of body weight, half the dose being given in the morning and half in the evening. Boughton (1941) noted that if calves infected with *E. bovis* were given 5 gm. daily for eight days, late in the incubation period, they did not pass such large numbers of cysts during the following months as untreated controls. Boughton and Davis (1943) found sulphaguanidine of value in calves naturally infected with coccidiosis: six calves were given 30 gm. every second week till a total of 150 to 180 gm. had been administered, 5 gm. being administered twice daily for three consecutive days. As compared with six untreated calves of the same age, those that had received sulphaguanidine had the disease much less severely.

Sulphamezathine has been found to be active in bovine coccidiosis, but its cost of production is too high to warrant its extensive use. Excellent results have, however, been obtained by Steward

(1947a) in four outbreaks by the use of 4 : 4′-diamino diphenyl sulphone. The drug is given for six days at the rate of either 0·04 gm. per kgm. in twenty-four hours (0·5 dram per cwt.) or 0·08 gm. per kgm. in forty-eight hours (1 dram per cwt.), six or three doses being given during the six days, according to the dosage employed. In either case the first loading dose should be double the following doses. In successfully treated calves the blood level must be maintained at from 2 to 3 mgm. per 100 ml. Symptoms promptly clear up and egg counts decrease rapidly, *E. zürnii* disappearing before the other larger species *E. bovis*. In order to eliminate infection it is advisable to treat all cattle in the herd which may have become infected.

Knappenberger (1949) still prefers copper sulphate and iron sulphate, 4 gm. and 8 gm. respectively, dissolved in 1·13 litres (1 quart).

In **lambs,** Foster *et al.* (1941) showed that a daily dose of 2 gm. sulphaguanidine prevented infection if given at the same time as an infecting dose, while it reduced an already acquired infection. A daily dose of 1 gm. reduced cyst formation during the time it was administered and did not interfere with the growth of the lambs.

Sulphaguanidine in a dose of 1 gm. per 10 lb. of body weight was found by Alicata and Willett (1946) to reduce the oocyst output in swine infected with *Eimeria debliecki* and *E. scabra* and to prevent the onset of toxic symptoms.

In naturally infected **puppies** sulphaguanidine in a dose of 1 gm. per lb. of body weight had little or no action (Whitney and Whitney, 1941). Sulphanilamide was used by Stoddard (1943) in infection due to *E. canis*. Parkin (1943), however, had more success with enemata of sodium sulphanilyl sulphanilate in dogs infected with *Isopora bigemina*, *I. rivolta*, *I. felis* and with one species of *Eimeria*. The enema, which was repeated after twenty-four hours, was made up of 10 ml. of a 1 per cent. solution of sodium sulphanilyl sulphanilate per kgm. of body weight. The fæces were as a rule free from oocysts in forty-eight hours. One cat was also successfully treated. No toxic symptoms were noted. Steward (1947b) has shown that in dogs with an initial dose of sulphamezathine of 0·1 gm. per kgm. of body weight followed by 0·05 gm. per kgm. (0·35 gr. per kgm.) twice daily a blood level of 3 mgm. per

100 ml. can be constantly maintained. Infections in cats were also found by Brumpt (1943a and b) to react to injections of mepacrine, 0·01 gm. per kgm. of body weight being the usual dose.

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Coccidiosis in Man

Human coccidiosis due to *Isopora belli* is such a rare condition, and when it does occur often causes so few symptoms, that its chemotherapeutic treatment has received but little attention. However, bismuth, iodine, quinine, gentian violet, emetine and anthelminthics have all been used without conclusive evidence as to their value. Three cases have been described by Quérangel des Essarts *et al.* (1946) in which, so it is claimed, mepacrine was efficacious in eradicating the parasites in doses of 0·3 gm. for five or six days. In two cases symptoms of diarrhœa were not affected by the mepacrine but were relieved by the administration of sulphaguanidine. Mepacrine with emetine bismuth iodide (0·13 gm.) and chiniofon for seven days eradicated the parasites in one case but failed in another, though symptoms disappeared (Logan, 1946); on the other hand, emetine bismuth iodide, 0·2 gm. nightly for twelve days, cured a third patient. In other cases, such as that recorded by Kiskaddon and Renshaw (1945), parasites disappeared spontaneously, and succinyl sulphathiazole improved the colitis.

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TRICHOMONIASIS

Trichomonas infections in human beings and in cattle present problems of considerable interest. In the veterinary field very little has been done from the therapeutic standpoint, but in the human disease curative procedures are legion and therefore largely empirical. The ideal trichomonicide has yet to be found for, as the Council on Pharmacy and Chemistry of the American Medical Association has emphasised (1943), with our present knowledge of the subject there are difficulties in setting up satisfactory criteria of treatment and cure. Failure to evolve a satisfactory chemotherapeutic drug is, however, somewhat surprising, as the cæcum of the rat is normally densely populated with trichomonad organs, *Trichomonas muris* and *T. parva*, and a suitable laboratory animal is thus provided for extensive therapeutic trials.

Liston and his colleagues (1947) point out that in women during pregnancy the pH of the vagina is about 5·3 and the concentrations of total lactate average 0·02 M. After parturition glycogen is absent, the pH is 6·0 and the total lactate falls to a very low level (average 0·006 M.). Conditions are then no longer favourable for the continued growth of *T. vaginalis*.

Hegner (1923, 1924 and 1933) found that high protein diets decrease the number of trichomonads in the cæca of rats but do not eliminate the parasites. Ratcliffe (1929), in a systematic study of the action of alkyl resorcinols, showed that the activity of these drugs on rat trichomonads increased with the length of the alkyl chain. Butyl-, hexyl-, heptyl- and octyl-resorcinols given orally to rats over a period of fifteen days, the total dose being 1·5 gm. per rat, reduced the numbers of trichomonads in the cæca. Heptyl- and octyl-resorcinols eliminated trichomonads in four out of ten rats and seven out of ten rats respectively. Hegner and Eskridge (1935) cured rats of their trichomonad infections by oral administration of carbarsone, a result which was confirmed by Gabaldon (1936a), who was able to cure rats regularly by oral, intravenous or subcutaneous administration of carbarsone. As a rule, multiple doses of the drug were given, the oral M.T.D. of carbarsone, according to Gabaldon (1936b), being in excess of 6,500 mgm. per kgm. Nelson and Tatum (1938) obtained very similar results with carbarsone. They also tested the effect of a number of other arsenicals on rat trichomonads, both *in vivo* and *in vitro*. Carbarsone was effective in tolerated doses when given by the intramuscular route but not when injected intravenously.

Acetarsol was found to be effective in rats as well as in infections due to *Trichomonas vaginalis* in man (Gellhorn, 1933). Tryparsamide appeared to be a far more satisfactory drug for the treatment of rat trichomoniasis than carbarsone, though the drugs possess an identical chemical structure except for the presence of a methylene group attached to the amine group in tryparsamide. Oxophenarsine was valueless by mouth, but the substitution of an hydroxyl group by OCH_2CH_2OH produced a chemotherapeutic index of 25 to 30 when the drug was given orally. Quinquevalent arsenicals were more effective because less toxic than tervalent arsenicals, and the frequency of free amine or amide groups in the more active quinquevalent compounds is noteworthy. Chiniofon showed some action on rat trichomonads, but quinine and emetine hydrochloride were valueless (David *et al.*, 1933).

In human infections due to *Trichomas vaginalis* a very large number of drugs has been used. Acetarsol was employed by a number of observers (Gellhorn, 1933 ; Haupstein, 1935 ; Bland

and Rakoff, 1936; Collis, 1936, and Assinder, 1936). Tablets composed of acetarsol and boric acid, and with a carbohydrate hydrolysed by a special process, have been found superior to vaginal douches; two tablets are inserted once or twice daily high in the vaginal fornices. In some cases dilute potassium permanganate or alkaline douches have been used in association with acetarsol. The majority of workers find that by far the best results are obtained in in-patients. Treatment must be continued for from two to three months, otherwise relapses are almost certain to occur (Assinder, 1936). Lloyd (1945), who treated 479 cases, thirty-two of them complicated with gonorrhœa, insists on the following routine: one tablet of 0·06 gm. is inserted into the vagina for eight weeks and then once daily during the next three menstrual periods. Once a week the vagina is irrigated with a solution of sodium bicarbonate (1 drachm to 1 pint), dried, and swabbed with Bonney and Browning's solution (crystal violet and brilliant green, 0·5 per cent. w/v of each in equal parts of alcohol and water). This proceeding is repeated at least once during two consecutive menstrual periods. Acetarsol and picric acid in kaolin have been used by Pérez *et al.* (1940). Toxic reactions, more especially skin rashes, and even exfoliative dermatitis, are not uncommon (Campbell, 1937; Long, 1937).

Carbarsone was found by Bland and Rakoff (1936) to be more effective than acetarsol when used to insufflate the vagina, 0·5 gm. of carbarsone being diluted with kaolin before use. Of 100 women thus treated, ninety-one were free from symptoms for from three to nine months, while of twenty-five women treated with acetarsol only twelve remained free from infection. Like all quinquevalent arsenicals, however, carbarsone is capable of producing toxic reactions and therefore search has been made for other less poisonous substances.

Chiniofon was recommended by Janeway (1935), a 4 per cent. solution being painted on the vagina at first twice and later once a week, then once before and once after the menstrual period; of twelve cases, ten were cured but two relapsed.

Vioform was first used by Huffman (1935), who treated fourteen females with a 6·6 per cent. suspension in glycerin and cured them all. Zener (1937) employed an ointment in thirty-eight cases and later (1939) in a further seventy-one cases: apart from three patients who discontinued treatment, 104 were cured and two failed to respond either to vioform, carbarsone or silver picrate: seven patients relapsed. A vioform ointment is not, however, easy to apply. A powder consisting of vioform 1 part and magnesium trisilicate nine parts was therefore applied to the vagina thrice weekly by means of an insufflator, about 50 gm. being required for each patient. Of 140 patients thus treated 137 were cured, a cure being looked on as having occurred when the patient had completed two menstrual cycles without recurrence, no clinical symptoms were present and no trichomonads were visible in smears (Angelucci, 1936; Adair and Hesseltine, 1936). Peterson (1938) reported the treatment of 500 patients with a paste containing 15 gm. of vioform in 22 ml. of glycerin: there was but one failure despite the fact that seven patients had resisted other forms of treatment. Vioform has so far produced no toxic results when applied to the vagina. A preparation, "trycogen," containing vioform, sodium thiosulphate, thymol and oil of wormwood in a base of boric acid, starch and magnesium carbonate has been used by Woodhull (1942) in 101 cases: only seventy-eight patients were cured.

Diodoquin has been employed by Owen (1941) in association with dextrose, lactose and boric acid, and by Karnaky (1940) under the term "floraquin." The vagina is first washed with 5 per cent. acetic acid, 1 to 2 drachms of the floraquin are then inserted and one tablet is subsequently used night and morning: among 4,400 patients, 94 per cent. are said to have been cured. Skin rashes may result from the intravaginal application of floraquin (Gaul, 1944).

Silver picrate (picrotol, picragol, silver trinitrophenolate) has also been used in the treatment of vaginitis due to *Trichomonas vaginalis* (Shelanski, 1936; Winther, 1936; Golub and Shelanski, 1937; Furnell, 1938, and Corbit *et al.*, 1941); it is employed in the form of either a 1 or 2 per cent. solution, as a compound powder, containing 1 per cent. in purified kaolin, for insufflation, or as a vaginal suppository containing 0·13 gm. in a boro-glyceride gelatin base (Buxton and Shelanski, 1937). Mascall (1937) found that in twenty-six out of twenty-eight cases *T. vaginalis* had disappeared from the vagina within twenty-four hours after a single insufflation of 0·3 gm. of silver picrate powder. It is advisable, however, to combine the insufflation with daily dry swabbing and the insertion of a pessary containing 0·12 gm. of silver picrate; the pessary treatment is continued for six days and is then followed by another insufflation: no douching is allowed during the treatment and no treatment is given during the menstrual period. Silver picrate is liable to cause desquamation of the vaginal mucosa and of the glans penis (Zener, 1939; Karnaky, 1937). Protracted use of this compound over a long period might possibly give rise to argyria because of its silver content, and nephritis because of its picric acid content. The urine should therefore be examined for albumin and casts and the skin studied for signs of pigmentation. Silver picrate *in vitro* is able to inactivate *T. vaginalis* in a dilution of 1 in 17,000. Suppositories containing 1 per cent. of picric acid are said to have produced good results (Angelucci, 1936).

Argyrol has been used by Reich *et al.* (1947) in the form of a powder containing 20 per cent. of pulverised argyrol, 40 per cent. of kaolin and 40 per cent. of β-lactose. After drying the vaginal tract 2 to 4 gm. are used for insufflation and each night after a douche of vinegar and water a gelatin capsule containing 4 gm. of the powder is inserted on seven days. By this treatment eighty-two of eighty-four negro and sixty-eight of sixty-nine white women are said to have been cured.

Propamidine was employed by Hanschell (1943) in the treatment of two cases of vaginitis. After cleansing with dettol and saline, two insufflations of propamidine powder were followed by the rapid diminution of purulent discharge and inflammatory redness, while Trichomonas disappeared and did not recur during the following four weeks.

Sulphonamide compounds have as a rule proved inactive in infections due to *T. vaginalis* (Allen and Baum, 1943): sulphathiazole, for instance, was found inactive *in vitro* in a dilution of 1 in 1,000 by Trussell and Johnson (1944), but in a jelly base it possessed some activity in a 20 per cent. concentration *in vitro*. Angelucci (1945) used an ointment containing 15 per cent. sulphanilamide, 2 per cent. allantoin and 5 per cent. lactose in a greaseless base, buffered to a pH 4·5 with lactic acid. About 10 gm. of the ointment are applied night and morning, and as the ointment is odourless, non-staining, non-irritating and spreads easily over the vaginal and vulvar surfaces, its use does not entail tampons or vulval pads. Improvement was obtained in ninety-eight of 100 cases. Of fifty-two cases followed up for more than six months eight relapsed.

Pantothenic acid analogues. Pantothenic acid is necessary for the multiplication of *T. vaginalis.* It was therefore hoped that an analogue of pantothenic acid might interfere with the use of this compound and inhibit its growth by competition. An analogue, (+)-α, γ-dihydroxy-β, β-dimethyl-N-(2-phenylmercapto)-ethyl-butyramide, kills *T. vaginalis in vitro* in a dilution of 1 in 800,000, according to Johnson and Kupferberg (1948), and crystalline and other fractions are each lethal at twice that concentration. *T. fœtus* and *T. gallinæ* are also said to be very susceptible *in vitro. In vivo* tests in monkeys and man show that this drug fails to eradicate infection. Streptomycin, 0·5 gm. suppositories every six hours for five to ten days was effective (Greenblatt and Wesr, 1949).

Nivaquine has, it is claimed (Boucher *et al.*, 1948), cured a severe case of cystitis due to *T. vaginalis.*

Detergents. Macdonald and Tatum (1948) have been specially impressed with the action of certain detergents such as aerosol OT, Dreft, Phemerol and Zephiran, which are able to cause dissolution of motile trichomonads even in the presence of blood plasma or mucin. Whereas *T. vaginalis* is killed by detergents in high concentration, *T. fœtus* is much more resistant. It is of interest to note that Siegler (1946), in reporting good results in the treatment of vaginitis and cervicitis, including *Trichomonas* vaginitis, used a sulphathiazole jelly composed of 87 per cent. of detergents.

Penicillin has no effect on *T. vaginalis* and, in fact, has been used as an aid to the isolation of the organism in pure culture (Johnson *et al.*, 1945). Weinman (1943) found that concentrations of tyrothricin containing 50 mgm. per ml. were fatal to *T. vaginalis in vitro*, the active substance being in all probability the tyrocidine rather than gramicidin. In two patients given local treatment with 0·5 mgm. of tyrothricin *T. vaginalis* duly disappeared, but relapses occurred after a short interval and the organisms were then entirely unaffected by further treatment with tyrothricin.

Œstrogens do not themselves have any direct action on trichomonas, but may be of help in the treatment of relapsing cases. Lloyd (1945), however, found stilbœstrol up to 5 mgm. daily for five weeks quite useless in a refractory case.

A solution of zinc chloride, 0·5 per cent., as a vaginal douche twice daily during the first week and once daily during the second week has been recommended by Novak (1946), but relapses are apt to occur.

Adair and Hesseltine (1936) used 95 per cent. lactose solution and 5 per cent. citric acid to supply the necessary nutriment for a normal vaginal flora. Kleegman (1930) preferred mercurochrome and Lassar's paste. Filler *et al.* (1942) have found negatan (negatol) of value ; this is described as a condensation product of *m*-cresol sulphonic acid and formaldehyde.

A considerable number of substances have been found to have an effect on cultures of trichomonas *in vitro*, though these results do not parallel those found *in vivo.*

Johnson and Trussell (1943) and Trussell and Johnson (1944), for instance, studied the effect of several compounds on bacteria-free cultures of *T. vaginalis.* All drugs were made up in 25 per cent. acidified human serum and allowed to act against 400,000 organisms in 4 ml. of fluid. The compounds, with the lowest concentration killing in ten but not five minutes, and the *p*H of the test mixture, are shown in the table on the opposite page.

By the technique employed, vioform showed some action as a 10 per cent. emulsion, but chiniofon and diodoquin were inactive. Acetarsol was lethal only in 1 in 20, carbasone only in 1 in 140 to 1 in 150.

MacDonald (1947) found that by *in vitro* tests *T. hominis* and *T. vaginalis* were similar in their reactions to drugs, but *T. fœtus* of cattle was more resistant. While salts of mercury were the most active, silver preparations, formalin and solutions of salts with high osmotic pressures were almost as effective. Mucin is liable to interfere with chemotherapeutic action.

Friedheim and Berman (1947) found that the condensation of oxophenarsine with BAL, resulting in the formation of 2-amino-4-

In vitro Action on *Trichomonas vaginalis*
(Trussell and Johnson, 1944)

Compound.	Dilution lethal in 10 minutes.	pH of test mixture.
Acriflavine hydrochloride	1 : 5,200	—
Acriflavine neutral	1 : 5,000	—
Proflavine	1 : 4,600	6·1
Arsphenamine	1 : 3,400	5·3–5·8
Neoarsphenamine	1 : 2,000	6·12
Sulpharsphenamine	1 : 4,000	6·0–6·2
Caprylic acid	1 : 1,000	5·0
Chromium trioxide	1 : 1,100	4·3
Copper sulphate	1 : 1,100	4·9
Dihexylin, aqueous	1 : 5,800	3·6
,, tincture	1 : 6,000	5·0
Ethyl mercuric chloride	1 : 42,000	6·3
Gentian violet	1 : 1,500	6·2
Malachite green	1 : 2,000	5·3
Mercuric bromide	1 : 15,000	6·4
Mercuric chloride	1 : 23,000	6·4
Mercuric oxycyanide	1 : 7,700	6·1
Merthiolate	1 : 13,000	6·7
Methyl violet 6B	1 : 1,500	6·5
Phemerol	1 : 1,600	5·9
Phenyl mercury derivate of sulphanilamide	1 : 26,000	6·4
Phenyl mercuric acetate	1 : 40,000	5·8
Phenyl mercuric benzoate	1 : 38,000	6·0
Phenyl mercuric chloride	1 : 38,000	6·0
Phenyl mercuric nitrate	1 : 38,000	1·9
Silver nitrate	1 : 30,000	6·3
Silver picrate	1 : 17,000	6·1
Strong silver protein (U.S.P. 8 per cent. silver)	1 : 1,400	6·2
Tartar emetic	1 : 2,000	2·4
Vuzin dihydrochloride	1 : 2,000	5·8

[methyl*cyclo*-(ethylenedimercaptoarsino)]-phenol in the form of the hydrochloride, enhanced trichomonicidal activity *in vitro.* Although *p*-melaminylphenyl sodium arsonate is only slightly active, the analogous antimony derivative *p*-melaminylphenyl sodium stibonate is highly active.

Jírovec and Peter (1945) also carried out *in vitro* experiments. According to their findings, chiniofon had some action, but mercury preparations were most active. Waksman *et al.* (1949) have found that an antibiotic, streptocin, isolated from the mycelium of *Streptomyces griseus* is very active *in vitro* against *T. vaginalis.*

A large number of substances are obviously able to kill *T. vaginalis* in low dilution, but the treatment of the actual condition is still in many ways unsatisfactory. How far an alteration in the *p*H of the vaginal secretion assists in bringing about cure by drugs is still uncertain. Many drugs are difficult to apply, stain the clothes and incommode the patient. Although a high percentage of cases may be cured with every treatment there remains a residue which resists all forms of chemotherapy at present available. In these cases, as recommended by Jírovec and Peter (1949), combined therapy is probably of value : in addition to a trichomonicidal agent, a bacterial agent such as a sulphonamide, a mycotic agent such as boric acid and lactic acid are added to encourage the growth of the lactic acid bacillus.

Intestinal trichomoniasis in man has received scant attention. Léon and Castillo de Léon (1942), however, have used an extract of *Lonchocarpus utilis* containing 4 per cent. rotenone. The drug was given in gelatin capsules and was followed by castor oil. Excellent results are said to have been obtained, and toxic symptoms were absent.

Trichomonads of Cattle. The treatment of *Trichomonas fœtus* infection in cattle is even more unsatisfactory than that of the human disease, despite the fact that serious economic loss may be inflicted on breeders, owing to abortion in cows and the infertility of valuable bulls. Lugol's iodine, potassium hydroxyquinoline sulphate (chinosol) 1 in 1,000, and sodium bicarbonate solution have all been recommended as vaginal douches. Earlier work is reviewed by Stableforth *et al.* (1937) and O'Dea (1939).

Swangard (1938 and 1939) and Dikmans and Poelma (1938) claim to have cured bulls by the application of a 0·5 per cent. acriflavine salve to the penis and mucosa of the prepuce, together with the injection into the urethra of 1 to 3 oz. of 0·1 per cent. acriflavine solution. The treatment is repeated after ten days, and must be given under an anæsthetic. Abelein (1938, 1941) has pointed out that these applications may cause some necrosis of the treated parts.

Stableforth *et al.* (1937) used 5 per cent. lactic acid as did Gould (1939): the crypt-like folds in the prepuce constitute a great difficulty in the use of a douche. Swangard (1938 and 1939), in cows, recommends enucleation of the corpus luteum and during the next few days vaginal douches with a 2 to 5 per cent. solution of sodium perborate: 100 ml. of 1 per cent. aqueous iodine solution is then injected into the uterus. Euler (1937) has also used iodine with success. More recently Bartlett (1946, 1948, 1949) used topical applications, ointments and douches of sodium hypochlorite and perborate, Lugol's solution, sodium dioctylsulphosuccinate and sodium ethyl mercurisalicylate without success. Trypaflavine also proved useless, but injections of sodium iodide, with potassium iodide by mouth, cured six of eight bulls. Sodium iodide was given intravenously to nineteen bulls with eight successes. Each dose consisted of 5 gm. per 100 lb. of body weight in 500 ml. of distilled water: five intravenous injections were given at intervals of forty-eight hours.

Better results were obtained by 0·5 per cent. of 3,6-diamino-10 methylacridinium chloride (trypaflavine neutral) and 0·5 per cent. of bis-2-methyl-4-aminochinolyl-6-carbamid hydrochloride (Surfen A) in a fat-free "washable" ointment base. Eight of nine bulls were successfully cured by two applications of this preparation, given at an interval of ten to fourteen days. The bulls must be given epidural anæsthesia: the genital membranes are then first washed with soapy water, dried and rubbed with 120 ml. of the ointment. The urethra must also be treated. Sippel *et al.* (1947) treated two bulls with intravenous injections of 44 gm. of sodium iodide per 1,000 lb. body weight at intervals of two days: five treatments were given with good results. Kerr (1942 and 1943) tested *in vitro* the killing action of a large number of substances on *T. fœtus*. The organisms were suspended in saline and in 1 per cent. peptone water. Brilliant green, gentian violet, acriflavine in 1 per cent. solution, and lactic acid were useless *in vitro* and far less active than iodine.

Many of the compounds recently tested in human infections do not appear to have been used in the bovine disease.

Culbertson (1940–41) was unable to find that either acriflavine or mepacrine had any action on trichomonads in the rat, *T. muris* or *T. parva*. The treatment of trichomoniasis in birds has long been unsatisfactory (David 1928; Spörri 1938). In the pigeon it has, however, been shown that acriflavine acts on *T. diversa*, while Bushnell and Twiehaus (1940) have used acriflavine in addition to ipecacuanha and catechu, sulphur, calomel and copper sulphate as treatment for turkey trichomonads. Knight *et al.* (1942) believe that gentian violet is a satisfactory treatment for the disease in turkeys. In trichomonad infections of pigeons Kraneveld and Nasoetion (1940) found sulphanilamide effective when given as an injection either intramuscularly or directly into the abscess in doses of 0·5 ml. of a 10 per cent. suspension every other day: local application as a paste was also of value, from 80 to 100 per cent. of pigeons being thus cured.

In infections due to *Trichomonas gallinæ*, the most commonly tried remedies, either as crop or mouth washes, or as injections, have been acriflavine, sulphonamides, copper sulphate and "sulfoliquid." Some apparent cures, some arrests and many failures have attended their application (Stabler, 1947a).

Stabler (1947b) found that 2-metanilamido-5-chloropyrimidine is valueless in chronic trichomoniasis of pigeons.

Copper sulphate added to drinking water has been extensively used in the treatment of trichomoniasis in pigeons: both favourable (Miessner and Hansen, 1936) and unfavourable results (Florent, 1938) have been recorded. Jaquette (1948), however, as a result of careful tests, came to the conclusion that the most effective concentration tested for non-breeding pigeons is 100 mgm. of the drug per 100 ml. of solution. The concentration most effective for breeding pigeons without producing evidence of toxicity is 35 mgm. of the drug per 100 ml. of solution. Concentrations of the drug greater than 100 mgm. per 100 ml. of solution for non-breeding pigeons and greater than 35 mgm. per 100 ml. of solution for breeding pigeons are markedly toxic, the symptoms being loss of weight, depression and possible liver damage.

The questions of the number of species of trichomonas pathogenic to birds and of the treatment of the infection require further study.

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GIARDIASIS

Although *Giardia intestinalis* is an inhabitant of the duodenum, there is no evidence that it ever invades the wall of the intestine, and the question of its pathogenicity is by no means certain.

As with *Balantidium coli*, a number of drugs have been used to eradicate the parasite, but although the protozoa disappear from the intestine the symptoms to which they are supposed to have given rise have not always been cured.

Acetarsol by mouth and injections of neoarsphenamine have occasionally been used (Bhattacharjea, 1943). Monat and McKinney (1946) employed acetarsol or carbarsone, the latter drug being given in a dose of 0·25 gm. four times a day for four days. More commonly mepacrine has been administered : this compound was first introduced by Brumpt (1937), who found that 80 per cent. of mice infected with *Giardia muris* were cured by the oral administration of a 1 per cent. solution of mepacrine. Later Galli-Valerio (1937) attempted the treatment of human cases with mepacrine, a treatment which has since been found to be effective by a considerable number of observers. In children under five years the usual daily dose is 0·1 gm., in children of five to twelve years of age 0·2 gm., and in older children and adults 0·3 gm. ; this medication is continued for four or five days (Tanguy, 1937; Heilmann, 1938; Martin, 1937 and 1938; Grüneis, 1938). Martin (1937) cured fifty-one of fifty-four cases, but Tanguy (1937) found two courses of five days necessary; one patient required four courses. De Muro (1939a) obtained better results by the oral administration of mepacrine than by intramuscular injections, though two cases were cured by this route. Chopra *et al.* (1939) reported that though mepacrine eradicated *Giardia, Trichomonas intestinalis*, *Chilomastix mesnili* and *Entamœba coli* were unaffected. Cain and Sikorav (1938) and others have emphasised the fact that mepacrine, while it removes the protozoa, cannot be relied on to cure the intestinal symptoms; it must, however, be remembered that mepacrine, when first administered, may give rise to intestinal discomfort in many healthy people. Nevertheless the results of treatment by mepacrine are extremely satisfactory. Nutter *et al.* (1941) treated twenty-three cases, and Hartman and Kyser (1941) a series of forty-six; of those followed up only one had relapsed. No toxic results have been described, but at first the stools may be more frequent. Harris and Mitchell (1949) treated a patient with 300 mgm. daily for ten days and cured not only the intestinal infection but an urticaria from which the patient had for long suffered.

Acranil, 3-chloro-7-methoxy-9-(2′-hydroxy-3′-diethylamino propylamino) acridine dihydrochloride, formerly known as sostol, was used for both adults and children by Grott (1938 and 1939), de Muro (1939b) and Weselmann (1943).

Berberian (1945) treated forty-five children and three adults, the dose being 0·1 gm. daily for five days in children from three to six years, 0·2 gm. daily in children of seven to twelve years, while in adults 0·3 gm. Occasionally the first dose was 0·2 gm. in children of seven years and 0·5 gm. in adults. Five weekly examinations failed to reveal parasites in the stools. As with mepacrine, acranil causes slight yellow discoloration of the skin, but no toxic effects have been noted. It has no effect on *Entamœba coli, Endolimax nana, Iodamœba* and *Chilomastix mesnili.*

Bose *et al.* (1944) used another butyl-acridine compound, 2-chloro-7-methoxy-5-(4′-diethylaminobutylamino) acridine. Two adults and five children were treated, the adults receiving 30 mgm.

daily for five days, the children 15 mgm. daily. No relapses occurred within an observation period of six months.

Schindel (1945), having failed to eliminate the parasites with 0·3 gm. mepacrine for five days, used diodoquin, 1·25 gm. daily, for ten days. The diarrhœa stopped in two days, and by the tenth day no more flagellates could be found in the stools.

In view of the effects of mepacrine, an acridine derivative, and other amino-acridines on *Giardia*, Culbertson (1940–41) studied the action of acriflavine on giardiasis in rats. Doses of 10 mgm. per 30 gm. of body weight for two days by mouth entirely eradicated the parasites from the intestine, there being nothing to choose between mepacrine and acriflavine. Both compounds had some action on *Hexamita muris*, but none on *Entamœba muris* or *Chilomastix bettencourti.*

Chloroquine, or " aralen " (4-(4′-diethylamino-1′-methylbutylamino)-7-chloroquinoline), has been used for the treatment of two patients with *Giardia* infection by Basnuevo and Sotolongo (1946) and Basnuevo (1948). The first dose for an adult was 1 gm. followed by 0·5 gm. on the second day and 0·25 gm. on each of the three following days ; the total dosage was 3 gm. : for a child the initial dose was 1 gm., the total dosage being 1·5 gm. Flagellates had disappeared from the intestine within two to five days of the end of treatment. Chloroquine had no effect on cysts of *Endolimax nana.* The follow-up was inadequate. Swartzwelder and Papermaster (1947) treated five children with chloroquine, but the parasites showed only a temporary disappearance after 250 mgm. daily for three days. Hambly (1948) also failed, for only three of fourteen patients given 2·2 mgm. per kgm. of body weight daily for seven days were cured. Schneider and Uzan (1947) used sontoquine (nivaquine), 3-methyl-4-(diethylamino-*iso*pentylamino)-7-chloroquinoline. Sixteen patients were treated, of whom five had failed to show any improvement with mepacrine. For adults and children over ten years of age, 0·3 gm. of the dihydrochloride was given for five days ; children under ten years received half that dose. Gastric disturbance was seen in one patient. Thirteen patients were cured by one course, two others by two courses, but a third failed to respond.

In the belief that failure to eradicate the parasites is due to their presence in the gall-bladder, a belief for which there is little foundation, Láng (1945) gave daily intravenous injections of 10 ml. of a 40 per cent. solution of urotropine for four to six days.

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CHAPTER VI

THE CHEMOTHERAPY OF LEISHMANIASIS

ONE of the most important of the earlier advances in chemotherapy was the discovery that antimony is of value in the treatment of visceral leishmaniasis. Before the introduction of antimonial therapy the death rate from kala-azar was almost 100 per cent.

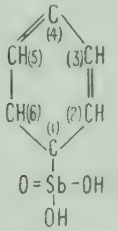
Three periods can be distinguished in the treatment of leishmanial infections; the first, when antimony compounds of the tartar emetic type were exclusively employed, the second characterised by the use of quinquevalent antimony derivatives, and a third period which begins with the introduction of the aromatic diamidines, compounds which at first appeared to be of particular value in Sudanese kala-azar since antimony salts often failed to control this form of leishmanial infection.

ANTIMONY PREPARATIONS

The credit for demonstrating the specific action of antimony in leishmanial infections is due to Vianna (1912), who first used tartar emetic successfully in the treatment of South American leishmaniasis. Confirmation of these results was quickly forthcoming from Italy where Di Christina and Caronia (1915) found antimony effective in the treatment of infantile kala-azar, and from India, where Rogers (1915), Muir (1915), Mackie (1915) and others used it with success. Antimonyl preparations of the tartar emetic type, however, are relatively toxic (p. 62), and in patients with visceral leishmaniasis are liable to cause broncho-pneumonia.

In 1911 the preparation of quinquevalent antimonial drugs became possible as the result of a method of synthesis discovered by Schmidt (1920). In this process, by the interaction of diazotised aniline and antimony trioxide in the presence of alkali, phenylstibonic acid is apparently formed.

An examination of the structural formula of phenylstibonic acid shows that the therapeutically active element, antimony, is directly united to a carbon atom, while in the tartar emetic series it is joined to carbon through oxygen.



Phenylstibonic Acid

A large number of compounds can be formed by substitution of one or more hydrogen atoms in positions 2 to 6 in the benzene nucleus, by Cl, or by groups such as amino or hydroxyl. In all these organic antimony compounds the antimony is not ionised in solution and therefore does not give the usual analytical reactions of "inorganic antimony" until the associated organic residue has been destroyed.

The quinquevalent antimony compounds so far prepared can be classed under four headings :—

(1) Salts of *para*-aminophenylstibonic acid :
  (*a*) Sodium *para*-aminophenylstibonate— "stibamine."
  (*b*) Diethylamine *para*-aminophenylstibonate —"von Heyden 693," "von Heyden 693B," "neostibosan," "stibosamine."
  (*c*) Urea salt of stibanilic acid—"urea stibol."

(2) Derivatives obtained by substitution in the amino group of *para*-aminophenylstibonic acid :
  (*a*) Sodium *para*-acetylaminophenylstibonate — "stibacetin," "stibenyl."
  (*b*) Urea and glucose combined with *para*-aminophenylstibonate—"amino-stiburea."
  (*c*) Nitrogen glucoside of sodium *para*-aminophenylstibonate—"neostam."
  (*d*) "Urea stibamine."
  (*e*) Sodium *para*-N-phenylglycineamide-*p*-stibonate.

(3) Derivatives obtained by substitution in the benzene nucleus of *para*-aminophenylstibonic or *para*-acetylaminophenylstibonic acid :
  Sodium *meta*-chloro-*para*-acetylaminophenylstibonate —"stibosan"—"von Heyden 471."

(4) Antimonyl compounds in which quinquevalent antimony is joined through oxygen to a chain of carbon atoms having a number of hydroxyl groups :—
  (*a*) Sodium stibogluconate—"sodium antimony gluconate," "solustibosan," "stibatin," "solusurmin."
  (*b*) N-methylglucamine antimoniate (2168 RP), "glucantamine."
  (*c*) Stibanose.
  (*d*) Sodium mannitol antimoniate.

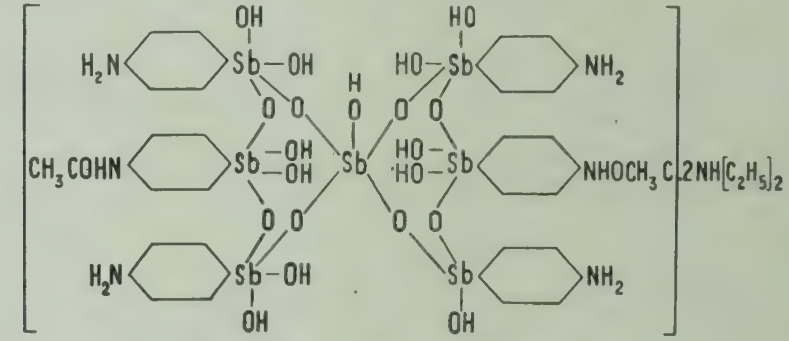
(5) An amino compound (Schmidt 1948).

The first of the quinquevalent antimony compounds to be tested clinically was stibacetin or stibenyl, the antimony analogue of atoxyl. This compound was used in Italy by Caronia (1916), and in the Mediterranean area its use has continued ever since: it is convenient since it can be given intramuscularly or even subcutaneously, but in India it proved quite ineffective and toxic reactions were common, possibly because it was unstable under tropical conditions. Fargher and Gray (1921) found that for mice the MLD was 133 mgm. per kgm. of body weight, compared with minimal lethal doses for sodium and potassium antimonyl tartrate of 25 mgm. and 16 mgm. respectively. Goodwin (1944a) reported that the LD 50 for mice was 5·65 mgm. per 20 gm., or 282·5 mgm. per kgm.

Urea stibamine was introduced by Brachmachari (1922). At first it was thought to be urea combined with *para*-aminophenylstibonic acid, but the work of Ghosh and his colleagues (1928) and of Gray *et al.* (1931) has shown that, as available in commerce, " urea stibamine " is not a single substance. The latter workers found that, in addition to a di-substituted urea, *s*-diphenylcarbamide-4 : 4′-distibonic acid, it contained antimonic acid, together with some *p*-acetylaminophenylstibonic acid, resulting from the incomplete hydrolysis of the stibacetin used as a starting substance. Owing to the weakly acid character of these materials they retain only a small amount of ammonia, which suffices, in consequence of their predominantly colloidal nature, to bring the whole into solution. The same or a closely allied product is available in India under the name of carbostibamide. The antimony content of various commercial samples of urea stibamine was found by Ghosh, Chopra and Chatterjee (1928) to vary between 20 and 43 per cent. Reed *et al.* (1946) reported that for one sample of urea stibamine from India the LD 50 was 404 ± 27 mgm. per kgm., the antimony content being 47·16 per cent. An American sample contained 43 per cent. of antimony and had an LD 50 of 266 ± 19 mgm. per kgm. Guha *et al.* (1943), in examining more than one hundred different samples, found that the usual antimony content was from 39 to 42 per cent.; in only five samples was a figure between 42 and 44 per cent. obtained while in only one sample was the antimony content below 39 per cent. The maximum figure obtained by Guha *et al.* was less than the minimum figure of Gray *et al.* (1931). The lethal dose for mice, according to Napier (1927), is 250 mgm. per kgm. of body weight, and the tolerated dose is 175 mgm. Guha, Dutta and Mukerji (1943) found with different batches that the maximum tolerated dose was 150 mgm. per kgm. of body weight, it seldom exceeded 170 mgm. The lethal dose for mice varied from 200 to 250 mgm. per kgm. of body weight. Goodwin (1944a) found the LD 50 for mice 4·26 mgm. per 20 gm. body weight.

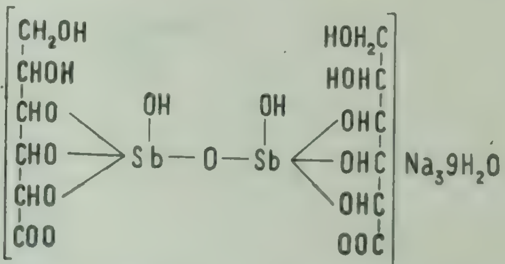
With an American sample of urea stibamine, Reed *et al.* (1946) noted in mice protrusion of eyeballs, hæmorrhage into the ocular tissue and opacity of the cornea.

Neostibosan was reported by Napier (1927) to be less toxic for mice as the minimal lethal dose was 350 mgm. per kgm. The formula is described by Schmidt (1948) as follows :—



Neostibosan (molecular weight, 1983·8).

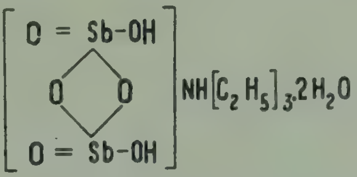
One disadvantage of neostibosan is that when dissolved in water it is not very stable. This was overcome by the production of solustibosan No. 561 (Schmidt, 1937), now known as sodium stibogluconate. The exact chemical constitution of compounds of this type cannot be easily determined, but Schmidt (1948) provides the following structural formula :—



Solustibosan (molecular weight, 907·6).

They may perhaps best be regarded as antimony pentoxide brought into solution by the pressure of an organic compound bearing hydroxyl groups. Sodium stibogluconate is a clear colourless solution which, curiously, does not give the serum precipitin test. Originally 1 ml. contained 20 mgm. of metallic antimony, while 1 ml. of a 5 per cent. solution of neostibosan contains 21 mgm. of antimony. More recently solutions have been prepared containing 50 mgm. of antimony per ml. (Patel, 1944) or even 100 mgm. per ml. These solutions are hypertonic but are said to cause little or no irritation (Kikuth and Schmidt, 1943). The rate of excretion in the urine of man is rapid, 68·5 per cent. being excreted in the first twenty-four hours, instead of 50 per cent. in the case of neostibosan. According to Weese (1937), mice survive the subcutaneous injection of 43 ml. or 860 mgm. of Sb per kgm. of body weight, while neostibosan kills 50 per cent. of mice when 615 mgm. of Sb is given. In the rabbit 15 to 18·5 ml. of sodium stibogluconate, 300 to 370 mgm. Sb per kgm. of body weight, leads to transient collapse. Intravenous injections up to 1·5 ml. per kgm. of body weight caused no pathological changes in the liver and kidneys of rabbits. Goodwin (1944a) found that the toxicity of this substance varies according to the method of preparation. A slight decrease in toxicity occurs on autoclaving.

An amino compound has been prepared by Schmidt (1948) with the following formula :—



Antimony amino compound.

In hamsters infected with leishmaniasis this compound is actively curative.

Oily suspensions containing 54 mgm. of quinquevalent antimony in 1 ml. have also been prepared (Kikuth and Schmidt, 1943).

The toxicity of a number of quinquevalent antimonials has been investigated by Goodwin (1944a). The LD 50, its limits and the slope of the regression line fitted to the observations upon the toxicity of each compound are shown in the table :—

TOXICITY OF ORGANIC QUINQUEVALENT ANTIMONY COMPOUNDS INJECTED INTRAVENOUSLY INTO MICE (Goodwin, 1944a).

Compound.	Total number of mice used.	b	σ	LD 50 mgm./20gm.	Per cent. limits ($P = 0{\cdot}95$).
Neostam	70	4·09	0·83	29·5	82–122
Neostibosan	90	6·50	1·03	9·44	89–112
Urea stibamine	120	1·29	0·16	4·26	64–157
Stibacetin	65	3·68	1·17	5·65	82–122
Sodium stibogluconate (i)	46	18·0	5·09	32·5	94–106
(ii)	80	9·78	2·20	33·0	93–108
Sodium mannitol antimoniate	70	13·0	2·57	102·2	93–107

The irritant activities of these preparations were determined by injecting solutions of graded concentration intradermally into the shaved flank of a guinea-pig and noticing the least concentration which produces a clear local reaction during an observation period of three days (Paget, Trevan and Attwood, 1934). Irritancy is related to toxicity and trypanocidal activity, but all three factors are independent of antimony content.

It is unfortunate that there is as yet no international standard of unvarying composition for antimony as for the organo-metallic compounds of arsenic.

Treatment with Quinquevalent Antimony

The value of quinquevalent antimonials in the treatment of kala-azar in India, China, and the Mediterranean region is now fully established. Of the many quinquevalent compounds which have been produced three only are now in common use, neostibosan, urea stibamine and compounds of the solustibosan type.

Neostibosan is prepared as a light brown powder which is freely soluble in distilled water. Solutions must be freshly prepared. For adults the drug is used as a 5 per cent. solution, but in children, to whom relatively larger doses are given, a 25 per cent. solution is employed. In adults the drug is given intravenously, in children intramuscularly into the buttocks, where it produces a slight local reaction; Debono (1947), however, in Malta, prefers the intravenous route: small babies can be injected into the jugular vein. With intramuscular injection of neostibosan the relapse rate is as high as 20 per cent. Adults usually receive an initial dose of 0·2 gm., rising to 0·3 gm.: in very debilitated individuals 0·1 gm. is probably a safer dose with which to begin. In young children injections are given three times a week on alternate days and relatively higher doses are required than for adults: an initial dose is 0·05 gm., increased by 0·05 gm. to the limit of tolerance, as shown by immediate vomiting. The average dose is 0·1 to 0·15 gm. in babies under one year, 0·2 to 0·25 gm. in those from one to two years, and 0·3 gm. in older children. In India eight injections on eight consecutive days are usually considered sufficient to establish a cure, but in China, and also in the Mediterranean zone, most workers prefer to give a course of twelve to sixteen injections. By the end of the course there is as a rule little apparent improvement but, after an interval of about fourteen days, the spleen begins to decrease in size and by the third week there is a gain in weight. Patients who are afebrile when treatment is begun frequently show a daily rise of temperature following each injection, and for the first few days of treatment there is often an exacerbation of symptoms.

In China, Struthers (1931) finds that an initial dose of 0·1 gm. is usually sufficient, but doses of 0·3 gm. are not as a rule well tolerated.

Urea stibamine is rather more toxic than neostibosan and causes so much pain that it should never be given intramuscularly. Instead of daily injections it should be given every other day or three times a week. The initial dose is usually 0·05 gm., followed by 0·1, 0·15, and 0·2 gm.; the maximum dose should exceed 0·25 gm. only in exceptional circumstances. Total dosage should not, however, exceed 2·5 gm., or very rarely 2·7 gm. Urea stibamine has been used with success not only in Indian cases of kala-azar but in those from the Mediterranean zone and from East Africa (Cole, 1944): it is now one of the most widely used antimonials in the treatment of visceral leishmaniasis.

Ho *et al.* (1949) found that in China the rate of disappearance of leishmanial parasites from the bone marrow depended on the numbers present in the marrow before treatment begins. Chen (1949), in China, treated twelve patients with total doses of 50 mgm. per kgm. in three days: one patient died.

Sodium stibogluconate (sodium antimony gluconate, stibatin) has the advantage that it can be given intramuscularly, intravenously or subcutaneously, and in addition it is not irritating. It is dispensed as a sterile isotonic solution which is said to remain stable indefinitely in sealed ampoules or rubber-capped bottles, containing an antiseptic. If the ampoule is opened it readily grows moulds and other organisms, since sodium gluconate is a good culture medium. Kikuth and Schmidt (1937) showed that in experimental leishmaniasis of the European hamster, *Cricetus frumentarius*, the amount of metallic antimony necessary to bring about a cure with sodium stibogluconate is larger than with neostibosan. Wang (1938) reported that in infected Chinese hamsters (*Cricetus griseus*) more sodium stibogluconate is required to bring about a cure than with either neostibosan or urea stibamine. Urea stibamine was the most effective of the three drugs. Wang (1939) found that subcutaneous injections of sodium stibogluconate for ten to twenty-five days, involving a total of 120 mgm. of antimony, produced a lower cure rate in Chinese hamsters than neostibosan, involving a total of 168 mgm. of antimony, for the same period. Daily injections of neostibosan or sodium stibogluconate were, however, less effective than bi-weekly or tri-weekly injections of sodium stibogluconate.

Tests on infected hamsters by Kikuth and Schmidt (1943) reported that while eight injections of the watery solution, involving the administration of 500 mgm. of sodium stibogluconate per kgm. of body weight (1,080 mgm. of antimony), sufficed to bring about a cure, the same result was obtainable by a single intramuscular injection of 6 ml. (324 mgm. antimony) of the oily suspension per kgm. of body weight. These results have not yet been confirmed. Pharmacological tests on mice proved that a mouse of 20 gm. body weight would tolerate a subcutaneous injection of 0·5 ml. of the oily preparation (1,350 mgm. of antimony per kgm. of body weight, as against 860 mgm. of antimony given as a watery solution). An X-ray examination of mice showed that the oil depot had been wholly absorbed within forty-eight hours.

In man, sodium stibogluconate has been used in China, in India, and on cases from the Mediterranean and Central Asian foci. Struthers and Lin (1937) treated twenty-nine patients, of whom three died during treatment, three failed to complete the course, one failed to respond and twenty-two were discharged as cured. Yates (1937) obtained cures in seventy-eight out of eighty-two patients. Five daily doses of the drug were well tolerated, 60 ml. per 100 lb. of body weight being administered. Chung *et al.* (1942) treated twenty-four cases by injections on alternate days, a total of from 60 to 162 ml. being used. Twenty-two cases were cured, one failed to complete the course and one failed to respond. In India, Napier, Chaudhuri and Rai Chaudhuri (1937) obtained excellent immediate results in ten cases, while Patel (1944) treated six, and Dastidar (1945) twenty-one cases. Patel's adult patients received ten daily injections each of 20 ml., a total of 400 mgm. of quinquevalent antimony, and three children received doses of 59, 120 and 150 ml. : six months later five patients were seen and found to be in good health. Burke and Chakravarty (1944) treated twenty-one cases with the dilute solution of sodium stibogluconate : doses were limited to 1 ml. per year of age up to the age of fifteen. As much as 100 ml. was given in seven days. All cases were cured.

Dastidar (1945) used total doses of only 60 ml. and reported immediate cures, but unfortunately did not follow up his patients. Choudhuri (1946) gave doses of 10 ml. for an adult weighing 8 stone (50·8 kgm.), the full course being not more than 200 ml. Of twenty-five cases, treated on alternate days either with intravenous (nine cases) or intramuscular injections (sixteen cases), twenty-two were cured. One died of cerebral malaria, one absconded and one required a course of urea stibamine.

Concentrated solutions of sodium stibogluconate can be given at intervals of twelve hours, a total of 0·4 ml. per kgm. of body weight being administered in ten injections in five days. The concentrated solution was first tested by Ramos Fernández *et al.* (1942).

Lozano Morales (1943) used the concentrated aqueous solution of sodium stibogluconate in five children with kala-azar in Spain. The injections were given into the buttocks on alternate days, the total dosage being 0·6 ml. per kgm. of body weight. The first two injections were given as a divided dose to avoid accidents due to hypersensitivity. For weights up to 30 kgm. the following dosage was recommended :—

Body weight.	First injection.	Second injection.	Subsequent injections.	Total.
10 kgm.	0·2 ml.	0·4 ml.	0·6 ml.	6 ml.
15 ,,	0·3 ,,	0·6 ,,	0·9 ,,	9 ,,
20 ,,	0·6 ,,	0·6 ,,	1·2 ,,	12 ,,
25 ,,	0·7 ,,	0·8 ,,	1·5 ,,	15 ,,
30 ,,	0·9 ,,	0·9 ,,	1·8 ,,	18 ,,

There was rapid improvement and no toxic reaction. either local or general.

While the usual dose of the dilute solution given to man was 2 ml. for each kgm. of body weight, or 40 mgm. of quinquevalent antimony per kgm. of body weight, it was soon apparent that a single dose of 10 or 12 ml. could be safely tolerated. As the quantity to be injected with these higher doses was large, clinical trials were carried out in man with the oily suspension. Although a single injection did not bring about a cure it was evident that this was effected by a considerably smaller number of injections of the oily suspension than of the watery solution of sodium stibogluconate.

Experiences in Spain with children showed that intramuscular injection of the oily suspension was well tolerated. both locally and generally. In over 90 per cent. of the cases treated early there was rapid response, the fever falling after the first injection. Only in severe cases, and in those which had resisted other treatment, was it occasionally necessary to resort to a second course.

Gil Bermúdez (1943) found that occasionally, when aqueous solutions of sodium stibogluconate failed to bring about a cure, the oily suspension was effective ; the suspension was given on alternate days intramuscularly, the total quantity representing 2 ml. for each kgm. of body weight. The oily suspension was such that 1 ml. represented 54 mgm. of antimony. Fernández Castanys (1945), in children aged from seven months to three years. also used an oily suspension of sodium stibogluconate : injections were made intramuscularly, from 10 to 19 ml. being given in from seven to ten daily injections. Further investigations are required to determine the relative value of the oily and aqueous preparations.

Lipscomb and Gibson (1944) reported a case contracted in Malta where no improvement occurred on neostam but 86 ml. of sodium stibogluconate given in fifteen days brought about a cure.

Kirk and Sati (1947) found that in the Sudan massive doses were effective in cases which failed to respond to more toxic antimony compounds. Particular interest attaches to the treatment with sodium antimony gluconate of Sudanese cases of kala-azar. Twenty-two patients were treated by Kirk and Sati with a preparation in which 1 ml. contained 75 mgm. or the equivalent of 20 mgm. of quinquevalent antimony, and later fourteen patients were given a preparation containing 100 mgm. of quinquevalent antimony per ml.

When treatment with the weak solution was limited to a course of ten daily injections of 6 ml., from two to four courses were necessary to produce a cure, but four of the twelve patients so treated died. In a further series of eleven patients treated with the weak solution, from twenty to forty injections were necessary to ensure cure. Intensive treatments also were undertaken. Two patients each given twenty injections in five days appear to have been successfully cured. The concentrated solution was used in fourteen cases with only one death in a patient who was almost moribund on admission to hospital, and with thirteen apparent recoveries, only six to ten injections being necessary. Toxic effects were negligible : in one patient treated with the weaker solution the second, third and fourth injections were associated with rigors. The only toxic symptoms with the concentrated solution were in one case a transient short cough and a feeling of tightness behind the sternum on administration of the first three injections.

Tuckman (1949) in China treated 148 patients with concentrated solutions : two died during treatment but of eighty-four followed for seven to twelve months only two relapsed and three died from other causes : seven had pneumonia. The best of the earlier results with solustibosan are those reported by Sati (1942), where the immediate cure rate was 77 per cent. with much larger total doses of quinquevalent antimony than are effective in Indian kala-azar. With the more intensive treatment possible with concentrated solutions, a cure can be obtained in Sudanese kala-azar in less than three weeks, in some cases in as short a time as five or six days ; this is a very remarkable result. Intensive therapy with more concentrated preparations of sodium antimony gluconate may well remove also that bugbear of Sudanese kala-azar, the " antimony-resistant case " which was believed to occur if the physician began treatment with small doses.

In somewhat striking contrast to these good results are those obtained by Maegraith *et al.* (1947) in treating eight patients, ex-servicemen who had been in India or the Mediterranean zone. The dilute solution of sodium antimony gluconate was used and it was intended to give 12 ml. twice daily for ten days. Only three of the patients were able to complete the course while one patient who tolerated a first course without incident had a severe febrile reaction during a second. Although six of the eight patients were apparently cured, in five cases severe rigors occurred with high fever after the tenth, twelfth or fourteenth doses. The appearance of the reaction was not related to the severity of the case before treatment, the geographical origin of the disease or the condition of the patient. In addition there was no relation between the size of the dose injected and the development of the reaction except that the patients who did not react received heavier doses of antimony than those who did. In one fatal case the organs examined at necropsy contained approximately the equivalent of two doses of the drug, but the histological findings did not indicate poisoning by antimony. It is difficult to find any reason why the results of the Liverpool workers should have differed so greatly from those of all the others who have used sodium antimony gluconate in other parts of the world. Reactions of an anaphylactic nature are very rare with quinquevalent antimonials, and according to Napier (1938) they have not been known to occur when injections are given daily. Chemical changes in the sample of drug used are unlikely since different batches produced the same result and the same batch did not produce rigors in all patients.

Following on the intensive use of antimony in the treatment of bilharziasis, Adams and Seaton (1947) tested the effects of intensive doses of sodium antimonyl tartrate in six cases of Indian kala-azar. A 2 per cent. sterile solution of the compound was used; the total dosage was 0.06 gm. per 12 lb. of body weight in six intravenous injections given at three-hourly intervals, usually at 9 a.m., noon, and 3 p.m., on two successive days. The appropriate dose was made up to 15 or 20 ml. with normal saline and injected intravenously through a fine needle, at least 5 minutes being taken over the injection. Most of the patients had paroxysms of coughing lasting about a minute, and in a few instances coughing was followed by vomiting. One patient developed a toxic jaundice two days after the injection. One case showed no response whatever to the drug, another relapsed after a short period of clinical improvement but was cured by a second course ; a third patient was observed for six months and was cured ; the three remaining patients showed no relapses over periods of observation of three, six and eight weeks.

The results of intensive antimony therapy do not compare with those obtained in India with neostibosan, urea stibamine or with the aromatic diamidines. The cost of treatment, however, is less than any of these, and the time, two days, taken for treatment compares favourably with eight days for neostibosan, twelve days for pentamidine and thirty days for urea stibamine.

Brief reference may be made to other antimony preparations used during the war years. In China, Chung and Chow (1942) reported on what is described as sodium mannitol antimoniate : this is a further example of antimony pentoxide in solution with an hydroxylated organic compound and is said to contain 21 per cent of metallic antimony ; it is dispensed as a 30 or 50 per cent. solution in 2 and 5 ml. ampoules and is thermostable so that it can be sterilised at 100° C. without decomposition. The solution can be given intravenously or intramuscularly. White mice are said to tolerate 0·3 ml. of the 50 per cent. solution intravenously, rabbits 20 ml. and dogs 40 ml. According to Goodwin (1944a), the LD 50 for mice by intravenous injection is 102·2 mgm. per 20 gm. of body weight.

In France, Giraud and Reyol (1943) have used *p*-aminophenylstibonate of N-methylglucamine (pentastib) : it contains 26·5 per cent. of metallic antimony and is made up in ampoules containing

0·5 gm. of antimony in 5 ml. It was originally prepared for veterinary use. The drug appears to be less effective than neostibosan or urea stibamine.

Sarrouy and Gillot (1943) preferred to give the drug intramuscularly rather than intravenously, as larger doses can be given and nausea and vomiting are less common: 0·1 gm. per kgm. of body weight was given daily for three days or 0·07 gm. per kgm. of body weight daily for four days.

In Tunis, Durand *et al.* (1946) used an antimoniate of N-methyl glucamine (glucantime, 2168 RP); they found that for adults twelve intramuscular injections of 20 ml., or 2 gm. of antimony, could be given in twenty-four days without signs of intolerance. This compound is very similar in its chemical structure to sodium stibogluconate and is said to contain 28·35 per cent. of metallic antimony: it dissolves readily in water, solutions being practically neutral. The injections were given every other day, thus allowing a larger amount of antimony to be administered in a given time than is usual in the therapy of kala-azar. For a child of four-and-a-half years of age the first injection was 2·5 ml., the second and third 5 ml. and the remaining injections 10 ml.; a total of fifteen injections was given.

Durand and his colleagues treated six patients with good results; three of these patients had relapsed after treatment with stilbamidine. Apart from a rise in temperature and some shivering after the injections there were no reactions. This compound has now been given to a number of patients, children and adults, in North Africa with good results (Naussac *et al.*, 1947; Sarrouy *et al.*, 1947a and b, and Athias *et al.*, 1947; D'Eshougues and Messerschmitt, 1948). In Italy, Carcassi (1948) used this compound in both children and adults. The temperature fell after the second injection. Janbon *et al.* (1949) reported a fatal case of cerebral hæmorrhage and œdema of the brain in an adult treated with N-methyl glucamine.

Toxic Reactions to Quinquevalent Antimony Compounds

Reactions to quinquevalent antimonials are not as a rule severe. Vomiting, coughing, headache, giddiness, flushing, faintness, and diarrhœa may occasionally occur, but usually only after a number of injections have been given and the maximum dose has been reached. With neostibosan, more severe symptoms may develop, usually at the same stage in treatment; the face becomes puffed, urticaria appears on the body, there is difficulty in breathing, with cyanosis, diarrhœa, vomiting, and sometimes loss of consciousness. With large doses of urea stibamine hæmorrhages may occur from the gums, nose and stomach, while retinal hæmorrhages have been seen and cerebral hæmorrhages have been suspected.

In China, Huang (1940) has drawn attention to the occurrence of agranulocytosis in kala-azar. Among 554 cases admitted to the Peiping Union Medical College Hospital twenty cases of agranulocytosis were due to kala-azar itself, but seventeen were attributed to the use of quinquevalent antimonials, neostibosan being incriminated in thirteen and urea stibamine in four, despite the fact that the latter compound was used more frequently and is more liable than neostibosan to produce headache, nausea, vomiting, and giddiness. Pentnucleotide appears to produce better curative results than blood transfusion. Although it has been found experimentally that BAL reduces the toxicity of both ter- and quinquevalent antimonials, its action in warding off toxic reactions in man has not yet been investigated.

For the avoidance of the more usual reactions it is essential to use double glass-distilled water and to give intravenous injections slowly; the drugs should not be too old, and after the injection the patient should lie down for half an hour. Adrenalin, ephedrin or pituitrin are of value in overcoming toxic symptoms, and the use of chewing gum overcomes the unpleasant taste.

Adrenalin, given in association with quinquevalent antimonials, does not increase the rate at which the spleen decreases in size (Aversa and Crosca, 1946).

It is well known that different batches of the same organic antimonial may show rather wide variations in toxicity. This question has been studied by Bose *et al.* (1946). In mice it was found that the toxicity of urea stibamine rose with an increase in the antimony content, but an increased antimony content is not the explanation of the varying toxicity. Some batches of urea stibamine with a high antimony content showed a low toxicity. The presence of antimonious acid, according to Bose and his colleagues, appears to be the real explanation of toxicity, for if the ratio of metallic antimony as antimonious acid to that present in the quinquevalent form in organic combination exceeds 1 : 26 there is a definite increase in toxicity. If care be taken in the preparation of urea stibamine to exclude antimonious acid the limits of toxicity are said to be much higher. It is doubtful whether this is a complete explanation for the toxicity of urea stibamine. The toxicity of neostibosan and certain other preparations is said by Bose *et al.* to depend on impurities in the amines used in their preparation.

There is some evidence that the toxicity of neostibosan can be decreased by incorporating in it a minute trace of a suitable reducing agent. *p*-Aminobenzoic acid apparently decreases the toxicity of quinquevalent antimonials for rats, but whether or not it interferes with leishmanicidal action is unknown (Sandground, 1943).

Dutta *et al.* (1946) have shown that when mice are not available pigeons can be used to determine the toxicity of organic antimony compounds : the drugs to be tested are injected intravenously into the wing vein.

Napier (1937), in India, reported that patients with kala-azar who were infected with tuberculosis failed to show any improvement when treated with antimonials : leishmaniasis combined with tuberculosis appears to be a very fatal combination. Struthers (1931), on the other hand, in China, believed that tuberculous patients improved as their leishmaniasis was cured. The question was investigated experimentally by Rose *et al.* (1946), who found that guinea-pigs infected with tuberculosis and treated with full courses of anthiomaline, stibophen, neostam or neostibosan did not develop a more rapidly fatal infection than control tuberculous animals untreated by any antimonials.

Ascorbic acid may possibly be of value as an aid to treatment by antimonials, for in children with infantile kala-azar Pinna (1947) has found a considerable decrease in the ascorbic acid content of the blood.

The Evaluation of the Leishmanicidal Action of Quinquevalent Antimonials

The evaluation of leishmanicidal drugs has been placed on a firm basis by the use of hamsters as experimental animals. Three species have been employed, the striped Chinese hamster, *Cricetus griseus*, the European hamster, *Cricetus frumentarius*, and the Syrian or golden hamster, *Mesocricetus auratus*. The last is probably the most satisfactory, since it readily breeds under laboratory conditions. Roehl (1929), using the European hamster, obtained a chemotherapeutic index of 1 : 5 to 1 : 7 for stibosan and 1 : 50 for neostibosan.

Kikuth and Schmidt (1937, 1938) examined liver smears stained for leishmania and thus first demonstrated the therapeutic activity of solustibosan. Van Dyke and Gellhorn (1946) tested the leishmanicidal activity of compounds by a very similar technique. Goodwin (1944b and 1945) evolved a somewhat more elaborate method in which portions of spleen were removed by biopsy immediately before and one week after treatment.. The number of parasites per 100 spleen-cell nuclei of all types was determined before and after treatment in dab preparations stained by Giemsa's method. From the data obtained in a series of animals treated with different doses of a standard drug, dose-response curves can be obtained which can be compared with those of a new drug under investigation. As there is found to be considerable variation in different tests it is essential to carry out parallel experiments with animals of the same stock treated with a standard preparation and with the drug under investigation.

Decreased protein intake lowers the resistance of hamsters to leishmaniasis. Repeated blockade of the reticulo-endothelial system produces similar results (Ritterson and Stauber, 1949).

Of the quinquevalent antimonials at present available, urea stibamine is more toxic than either neostibosan or solustibosan, but it is probably more curative than neostibosan. Of the relative curative value of urea stibamine and sodium stibogluconate it is as yet too early to speak, but there is no question that sodium stibogluconate, in concentrated aqueous solution or in oily suspension, is less toxic, and from the reports at present available quite as, if not more, effective therapeutically than other quinquevalent antimony preparations.

Whatever the compound used for treatment it is most essential that all patients should be kept under observation for eighteen months. Relapses may occur in from twelve to fifteen months after apparent cure.

The Mode of Action of Antimony Compounds

It has long been recognised that quinquevalent antimonials are far less toxic than tervalent preparations. This is probably due in part to the high rates of excretion of quinquevalent compounds, but the chemotherapeutic activity of the quinquevalent compounds, their possible activity in filarial infections, and their actual superiority over tervalent preparations in treating kala-azar have not been satisfactorily explained.

A direct leishmanicidal action is possible but is not probable owing to the low toxicity of quinquevalent antimonials for mammals, while a mere stimulation of the host's reticulo-endothelial system would hardly explain the highly specific action of quinquevalent antimony in kala-azar. By analogy with quinquevalent arsenic preparations it has been assumed that reduction takes place in the body to the tervalent state, the grouping stibinoxide (R—Sb = O) being parasiticidal.

Brahmachari *et al.* (1924) suggested that, as the initial excretion of urea stibamine was rapid and the remainder of the dose was excreted at about the same rate as tartar emetic, it followed that the slowly excreted fraction of the quinquevalent compound had undergone reduction.

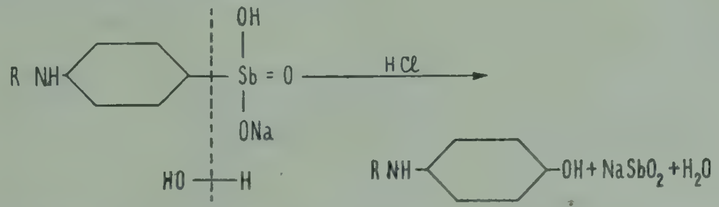
Bock (1927), on insufficient evidence, also considered that the low toxicity of stibophen was due to its being oxidised to the quinquevalent state in the body. Goodwin (1943), however, showed that urine collected from mice injected twenty-four hours previously with stibophen had the same trypanocidal activity as stibophen of equivalent antimony content. Quinquevalent stibophen had no trypanocidal activity. It was therefore concluded that no appreciable change in the valency of antimony had occurred. Using the polarographic method of estimating ter- and quinquevalent antimony, Goodwin and Page (1943) brought forward some evidence in favour of the view that reduction of quinquevalent antimony does occur in the tissues. This evidence is based on the following findings :—

(1) By incubation at 37° C. of normal rabbit's blood containing quinquevalent sodium antimony gluconate, equivalent to 0·001 gm. per 100 ml. of antimony, a small but significant increase of tervalent antimony occurs after seventy-two hours. Tissue cultures of chick embryo tissue incubated for twenty-four hours with quinquevalent antimony also cause a rise in tervalent antimony.

(2) The antimony excreted by man in from twelve to forty-five hours after an injection of quinquevalent sodium antimony gluconate showed appreciable amounts of tervalent antimony.

(3) Estimations of the tervalent antimony in the livers of rabbits and mice receiving injection of quinquevalent sodium antimony gluconate showed appreciable quantities of tervalent antimony.

It has been suggested by Gray (1943) that the hydrolysis of the *p*-aminophenylstibonic acid derivatives probably takes place in acid solutions *in vitro* as follows :—

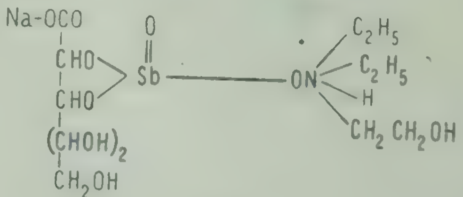


Treatment of stibamine glucoside with hydrochloric acid, followed by neutralisation with sodium hydroxide, doubles the toxicity of stibamine glucoside as compared with the untreated substance to which an equivalent amount of sodium hydrochloride has been added. This is further evidence that the toxic tervalent compound has been formed *in vitro*, but there is as yet no evidence to suggest a similar hydrolysis in the body. Nevertheless there are difficulties in accepting the theory that chemotherapeutic activity is dependent solely on the reduction of the quinquevalent to the tervalent form.

It is possible that tervalent antimony compounds are more toxic because the antimony in this valency can readily combine with the sulphydryl groups of intracellular enzymes whereas the antimony of quinquevalent compounds must first undergo reduction to SbIII. This theory might account for the higher toxicity of tervalent compounds, but further light might be thrown on the question if methods similar to those employed in the case of copper chelate (Calvin and Wilson, 1945) could be devised for determining the extent to which in the body antimony dissociates from the parent molecule.

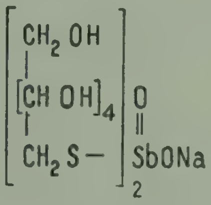
The fact that BAL reduces the toxicity both of ter- and quinquevalent antimony compounds suggests that the mode of action of antimony is similar to that of arsenic.

Gellhorn and van Dyke (1946) have attempted to correlate leishmanicidal action with distribution of antimony in the tissues and with chemical structure. Thus they found that there was partial correlation between the concentration of antimony in the spleen and the action on *Leishmania donovani*, as judged by parasite counts in liver smears. If, as with tervalent antimony preparations, there was a low concentration of antimony in the spleens of hamsters there was poor chemotherapeutic action ; the reverse, however, was untrue for stibacetin gave a high concentration of antimony in the spleen but no chemotherapeutic action. Others, however, have found that stibacetin is not entirely inactive. In examining the active and inactive quinquevalent antimony compounds it is noteworthy that they can be placed in two groups, Group A consisting of substituted phenylstibonic acids, Group B of quinquevalent antimonials in which the metal is linked twice through oxygen or sulphur to carbon. The active compounds in group A are neostibosan, neostam and probably stibacetin. It is suggested that these compounds owe their activity to the presence of free or potentially free *p*-aminophenylstibonic acid, the activity being lost if the *p*-amino group is acetylated. The only member of Group B which is therapeutically active is stibanose



Stibanose

Stibanose is a stibonic acid but the stibonic acid is not linked directly to carbon but indirectly twice through oxygen. In such a compound as sodium antimony thiosorbitol, the antimony is bound more firmly to the organic moiety because of its linkage



$$\left[\begin{array}{l} CH_2OH \\ | \\ [CHOH]_4 \\ | \\ CH_2S- \end{array}\right]_2 \begin{array}{l} O \\ \| \\ SbONa \end{array}$$

through sulphur; the rate of transference of antimony from drug to parasite is thus possibly very slow.

Sodium 4-acetyl aminophenylstibonyl gluconate and sodium 4-sulphonamidophenylstibonyl gluconate are inactive; they differ from stibanose in that the electrovalence is replaced by a covalence.

Whether or not the reticulo-endothelial cells of the body are stimulated by antimony to destroy the leishmanial parasites is still uncertain. There is, however, no evidence that the reticulo-endothelial cells of the body become loaded with antimony; stimulation, however, need not be accompanied by storage of the stimulating substance. As Wang (1940) has shown, the first action of neostibosan on the spleen of the hamster is to bring about the destruction of the parasites within the cells; later these cells undergo degenerative changes and die. There is no evidence of increased phagocytosis. Napier and Krishnan (1931) suggested that an important factor in ensuring a cure is the immunity response of the individual patient, while Boix Barrios (1943) believes that so-called antimony-resistant cases are resistant not because of any peculiarity in the parasite but because the reticulo-endothelial cells fail to react to antimony. This explanation would hardly account for the failure of Sudanese cases to react to antimony except when large doses of compounds of the type of sodium stibogluconate are given, or to the fact that, according to Soong and Hou (1944), strains of leishmania obtained from dogs in China and maintained in Chinese hamsters are resistant to antimonials which promptly cure hamsters infected with strains obtained from Chinese patients. Obviously there are strain differences in relation to sensitivity to antimony. A full account of the chemotherapeutic action of antimony up to 1938 is given by Schmidt and Peter.

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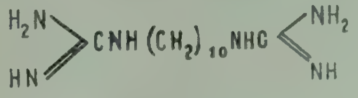
AROMATIC DIAMIDINES

In 1939, Ewins and his colleagues prepared a large number of aromatic diamidines (Ashley, Barber, Ewins, Newbery and Self, 1942), as a result of preliminary investigations fully described by Yorke (1940). In 1935 Jancsó and Jancsó (1935) had shown that synthalin (decamethylene diguanidine) cured trypanocidal infections in mice; this they attributed to its hypoglycæmic effect, since trypanosomes require carbohydrates for their metabolism.

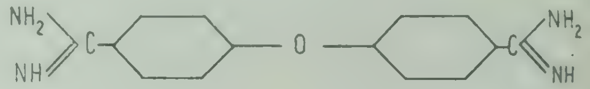


$$\begin{matrix}H_2N\\HN\end{matrix}\!>CNH(CH_2)_{10}NHC<\!\begin{matrix}NH_2\\NH\end{matrix}$$

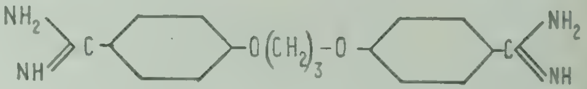
Synthalin

It seemed, however, so improbable that hypoglycæmia alone could destroy trypanosomes *in vivo* without also affecting the host that the whole question was reinvestigated by Lourie and Yorke (1937), who found that synthalin had a direct *in vitro* trypanocidal action at 37° C., independent of its hypoglycæmic action. The trypanocidal activity of synthalin was shown to be of the same order as that of the aromatic tervalent arsenicals. King, Lourie, and Yorke (1937) then studied the trypanocidal properties of a long series of guanidines, isothioureas, amidines and amines with alkyl and alkylene chains. Certain diamidines exerted a powerful trypanocidal action *in vivo* and *in vitro*, the most active being undecane-1 : 11-diamidine which produced cures in mice and rabbits infected with *Trypanosoma rhodesiense*. Ashley *et al.* (1942) extended the investigation to aromatic diamidines. Four groups of compounds were prepared : (1) compounds linked by a straight —C— linkage, (2) compounds linked by an —O— linkage, (3) compounds linked by —N— linkage, (4) compounds linked by an —S— linkage. Lourie and Yorke (1939) found that most compounds in the first three groups were active against trypanosomes but the most active compounds were :—

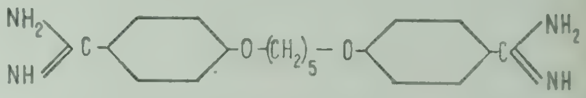
4 : 4′-diamidino diphenyl ether (phenamidine)



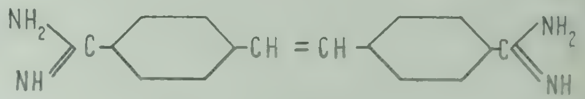
4 : 4′-diamidino 1 : 3 diphenoxy propane (propamidine)



4 : 4′-diamidino 1 : 5 diphenoxy pentane (pentamidine)



4 : 4′-diamidino-stilbene (stilbamidine)



These compounds are not only active against trypanosomes (p. 413) but against *Babesia* (p. 238) and *Leishmania*.

In place of pentamidine dihydrochloride, pentamidine diisethionate, 4 : 4′-diamidinodiphenoxypentane di-β-hydroxyethanesulphonate is now more frequently used. It is given intramuscularly and appears to be less toxic than the hydrochloride.

The pharmacological action of the chemotherapeutically active aromatic diamidines was investigated by Wien (1943) and Wien, Freeman and Scotcher (1943). The LD 50 and maximum tolerated doses are shown in the table. The toxic symptoms in mice, as described by Lourie and Yorke (1939), are narcosis, dyspnœa and tremors. Wien (1943) noted that with all four compounds there was general depression of the central nervous system; at first the respiration was quickened, later it became slow and forced. Slight ataxia was noticed in the hind legs while the rectal temperature fell. Death was due to respiratory failure. In rabbits, collapse was associated with cardiac and respiratory failure. The respiration, although initially stimulated, was later depressed, becoming slow and shallow: in some cases there were short periods of apnœa. Respiratory movements became difficult, as there was resistance to the intake of air. The rabbits lay prostrate, some with retracted heads, while a few suffered from feeble clonic convulsions. Some evidence of chronic toxicity was obtained when doses of one-fifth to one-third the toxic dose were employed in rats. The growth of the animals was decreased, the site of injections was inflamed, and the ears, nose and feet were flushed owing to vasodilation. Occasional tremors were seen while the body temperature was decreased.

TOXICITY FOR MICE

Compound.	Intravenous administration LD 50 (mgm./gm.).	Subcutaneous administration LD 50 (mgm./gm.).	Maximum tolerated dose by intraperit. injection of mice (mgm. per kgm. of body weight).
4 : 4′-diamidino diphenyl ether dihydrochloride	0·050	0·120	0·0375
4 : 4′-diamidino-stilbene dihydrochloride .	0·031	0·180	0·05
4 : 4′-diamidino 1 : 3 diphenoxy propane dihydrochloride	0·042	0·055	0·0375
4 : 4′-diamidino 1 : 5 diphenoxypentane dihydrochloride . . .	0·028	0·064	0·05

The four diamidines all caused a transient fall of blood pressure, but phenamidine was less active than the others. These effects were reduced but not abolished by full doses of atropine. Solutions of stilbamidine which have been kept exposed to light for some days become more active and produce a greater depressor effect. Calcium chloride or calcium gluconate in doses of 10 to 15 mgm. or 50 to 100 mgm. intravenously in cats, prior to an effective dose of a diamidine, reduces the fall in blood pressure ; this appears to be a purely vascular action since in the intact animal the previous administration of calcium affords no protection against the toxic action of these compounds. In addition, perfusion and plethysmograph experiments in the rabbit, cat and dog showed that the fall in blood pressure must be accounted for chiefly by peripheral vasodilation. The effect of the four diamidines on the heart was small and transitory : low concentrations stimulated and high concentrations depressed the heart. The general action on the blood pressure was similar to that of ergotoxine, the effect of adrenalin on the blood pressure, uterus and perfused vessels of the rabbit's ear and cat's hind limb being reduced. The isolated plain muscle of the rabbit's intestine, of the guinea-pig's uterus and of the cat's uterus was stimulated by the compounds in high concentrations ; these effects were not abolished by atropine.

In the frog, doses of 400 mgm. per kgm. of body weight of stilbamidine, propamidine or pentamidine injected into the dorsal lymph sac caused progressive depression of respiratory and muscular activity. Death occurs in two to four hours, the heart stopping in systole. By the ligated leg technique a curare-like action on skeletal muscle can be demonstrated (Seager, 1947).

The effects resulting from the administration of guanidine compounds and certain alkylene diamidines on the blood sugar have been extensively studied by Blatherwick *et al.* (1927) and Bodó and Marks (1928). The hypoglycæmic action of synthalin is fundamentally different from that of insulin. The glycogen disappears from the liver but it is not used up or stored as muscle glycogen ; in addition, the hyperglycæmic response to adrenalin is abolished. Broom (1936), however, found that though the toxic and glycæmic properties of certain alkylene diamidines closely resembled those of the diguanidines, carbohydrate metabolism was not so readily affected. Wien *et al.* (1943) examined the effects of phenamidine, stilbamidine, propamidine and pentamidine on the blood sugar. Effects were produced only with doses approaching the toxic level; the main effect was a hyperglycæmia. Phenamidine had the smallest action, but propamidine was the only compound which caused a subsequent hypoglycæmia. Adrenalin hyperglycæmia was reduced by the previous injection of stilbamidine. In man, Wingfield (1941) found that the blood sugar fell in accord with the blood pressure. The four aromatic diamidines also had some action on the kidneys, as shown by an increase in the blood urea and non-protein nitrogen figures. In some cases raised levels were obtained with doses which did not influence the blood sugar. Devine (1940) had also shown that stilbamidine, in doses up to 15 mgm. per kgm. of body weight, caused a two-fold increase in the blood urea without producing any significant change in the blood sugar. By subcutaneous injection phenamidine caused less response than stilbamidine; propamidine and pentamidine were the most toxic, their action being about equal. In dogs and rabbits the serum calcium and serum potassium levels were both reduced in a few hours. No significant changes in the red or white cells of the blood were seen in experimental animals, except when lethal doses were given, when a terminal polymorphonuclear leucocytosis was seen.

Seager and Castlenuovo (1946) noted that in rabbits toxic symptoms were often delayed; thus after a single subcutaneous dose of 50 mgm. per kgm. of body weight death might occur in from one to ten days. In mice no such delayed effect was noted.

The excretion of stilbamidine in mice has been studied by Fulton and Goodwin (1945a). After subcutaneous or intraperitoneal injection the highest serum levels are attained within thirty minutes of injection; after intravenous injection the highest serum level is attained within a few seconds. Two hours later the amount of stilbamidine in the serum is too small for detection. It seems that stilbamidine is adsorbed and stored by the tissues, but though it is readily adsorbed by cellulose or animal charcoal it is probably not adsorbed by red blood corpuscles.

The excretion of stilbamidine was investigated in rats by Wien (1946). The drug was injected subcutaneously into rats in doses of 1 to 10 mgm. per kgm. of body weight daily for fifteen days and the urinary excretion estimated by means of fluorescence, which measures the unchanged molecule, and by the glyoxal reaction which estimates both the unchanged molecule and the detoxified products if any. With a daily dose of 1 mgm. per kgm. of body weight the percentage excretion of the day's dose, as measured by fluorescence, was 1·6 on the fifth day, 1·8 on the tenth and 2·5 on the fifteenth day ; when estimated by the glyoxal method the results were 42 per cent. on the fifth day, 52 per cent. on the tenth day and 59 per cent. on the fifteenth day. A large proportion of stilbamidine was therefore presumably metabolised and excreted in a non-fluorescent form. Similar results were obtained when the excretion was followed of 2-amino 4 : 4′ diamidino stilbene, a compound which can be estimated by diazotisation as well as by fluorescence. After about five injections there was an optimum excretion of stilbamidine, that is, the greater the number of injections given the higher is the proportion of the daily dose excreted, while conversely the larger the daily dose above the therapeutic level, the smaller the proportion excreted. Presumably the kidney can eliminate only a limited amount of the compound. Rats exhibited some toxicity with doses of 5 to 10 mgm. per kgm. of body weight.

In man, Kirk and Henry (1944) estimated the excretion by the fluorescence method of Henry and Grindley (1945). Two Sudanese patients with kala-azar were given nine doses of 50 mgm. (1 mgm. per kgm. of body weight) intravenously on alternate days and the excretion in the urine determined. During the period corresponding to the first three doses only about 15 per cent. of the amount administered was found in the urine, then the excretion increased and by the seventh or eighth dose about 80 per cent. of the last dose was recovered. Within one day after the last dose 200 mgm. had been excreted out of a total dosage of 450 mgm. Within 15 minutes of the ninth or last dose the concentration of stilbamidine in the plasma was about 0·7 mgm. per 100 ml., and five days later none of the drug could be detected in the plasma. Within a few hours of the last injection no stilbamidine could be detected in the cerebrospinal fluid, sweat or sputum. Five days after the last dose traces were found in the urine ; similar traces were still present twenty-five days after the last injection. Stilbamidine thus again resembles mepacrine in being slowly liberated and in part excreted in the urine.

Further experiments on the excretion of stilbamidine, 4 : 4′-diamidino-2-aminostilbene, 4 : 4′-diamidino-2-iodostilbene and 4 : 4′-diamidino-2-hydroxystilbene were carried out, Hampton (1947), using a modification of the fluorimetric method of Henry and Grindley (1942) and the colorimetric method of Fuller (1944, 1945). The latter method showed a urinary excretion about twenty times greater than that estimated by the fluorimetric method. It is uncertain what is the nature of the non-fluorescent excretory products. The central double bond of stilbamidine may become saturated ; fission—reduction or oxidation—into two monoamidine residues may occur or the non-fluorescent metabolite may have a substituted group on one of the benzene nuclei of negative dipole moment.

The Estimation of Aromatic Amidines

A method for the colorimetric determination of stilbamidine was first elaborated by Devine (1944), based on the fact, discovered by Ekeley and Ronzio (1935), that certain aromatic diamidines, when heated with glyoxal in alkaline solution, yield yellow-coloured derivatives.

The original method was as follows :—

> Two ml. of stilbamidine solution, containing not more than 0·1 mgm. per ml., are mixed in a thin-walled test tube with 1 ml. of 10 per cent. glyoxal and 2 ml. of 3N NaOH, and heated in a boiling bath for thirty seconds. The tube is removed, cooled under running water for a few seconds and then in melting ice for five minutes. Finally 5 ml. of 15N H_2SO_4 (60 ml. of concentrated acid, diluted to 144 ml.) are quickly added with shaking ; the tube is warmed slightly in the hand to prevent subsequent moisture condensation on the cell during reading, and the solution is transferred immediately to an absorptiometer cell for determination, using No. 7 (dark blue) filters.

This method was modified by Fuller (1944), who rendered it more sensitive by heating in a borate buffer at *p*H 9. The modified test is sensitive for aromatic amidines in a dilution of 1 in 100,000. The buffer was prepared from 4 gm. boric acid neutralised in hot solution with caustic soda to *p*H 9 and diluted to 100 ml. The glyoxal reagent is used as the sodium disulphite in 0·5 per cent.

aqueous solution. When an estimation is to be made a few ml. of the amidine solution, roughly neutralised, are mixed with about 1 ml. of each of the reagents and the mixture maintained near the boiling point for a few minutes or left on the water bath for ten minutes, when a pink or magenta colour is produced. The best development of colour occurs with approximately two molecules of the reagents to one of amidine. The reaction is specific for an unsubstituted aromatic amidine group but does not take place with compounds in which one or two methyl groups are attached to the amino nitrogen of the amidine group : it does not occur with guanidines, biguanidines, amines or aliphatic amidines and is inhibited by excess of glyoxal.

Goodwin (1943) devised a spectrophotometric method which is about as sensitive as the original method.

A simple method has been described by Henry and Grindley (1942) to measure concentrations of stilbamidine as low as 0·025 mg. per 100 ml. in a few drops of fluid. The test depends on the fluorescence of stilbamidine in ultra-violet light. A rough estimation can be made by placing a No. 50 filter paper 1 cm. below a standard Dreyer pipette filled with the fluid to be investigated. One drop is allowed to soak freely into the paper and to dry. It is then compared under ultra-violet light with spots prepared from a series of known concentrations. A more orthodox chromatographic method may be used, the unknown fluid being allowed to percolate through a column of cellulose pulp. The sensitivity of this method, however, is low. The original method has been modified by Henry and Grindley (1945) to render it more sensitive. Hampton (1947) has also made slight modifications in the method.

Fulton and Goodwin (1945a) used a spectrographic method for estimating stilbamidine in biological fluids : about 2 ml. of serum is required and protein is first removed by dialysed iron. Manipulations must be carried out as far as possible in the dark, owing to the photolability of stilbamidine solutions. The method of estimation is based on the fact that stilbamidine in aqueous solution exhibits a strong spectral absorption band with a maximum at 329 mμ. The drug content of serum can be measured down to 5μgm./ml. with the limits of accuracy of a non-photoelectric spectrographic outfit (± 3 per cent.). Below a drug content of 5μ gm./ml. serum the accuracy of the method decreases owing to the preponderance of non-specific absorption.

Jackson *et al.* (1947) also used a fluorescent method for estimating aromatic amidines in plasma and urine. Fluorescent glyoxalidone derivatives are produced by reaction with glyoxal and benzaldehyde in alkaline solution. In calculating the concentration, the formula

$$x = \frac{S(I_x - I_b)}{(I_s - I_x)}$$

is used, where x = amidine in the original sample, S = amidine added to the standard sample, and I_x, I_s and I_b are the intensity readings of the original sample, the standard sample with added amidine, and the urine blank, respectively. The accuracy of the method as applied to pure aqueous solutions is about ± 4 per cent., while with plasma the average deviation of a single determination is about ± 6 per cent.

A simple field method for the estimation of diamidines in body fluids is based on the finding by Trought (1949) that Fearon's penta-cyano-ammonio-ferrate reagent gives a yellow to orange colour with distilled water solutions of the diamidines. A similar coloration occurs with the protein-free blood serum of patients who are being given therapeutic doses of the drugs. Standard calibration curves of the drugs are prepared and the unknown blood-serum diamide level can then be determined from these curves by means of a suitable colorimeter. A blank reading from the patient's serum before treatment is essential so that necessary corrections can be made in the readings from specimens obtained during treatment.

Treatment with Aromatic Diamidines

Treatment of leishmaniasis in man has been carried out with stilbamidine, pentamidine, and propamidine. Stilbamidine was originally employed as the hydrochloride which is not very soluble ; later the far more soluble diesthionate was used.

Stilbamidine. In the golden hamster *Mesocricetus auratus* repeated doses of 2·5 to 4 mgm. per kgm. of body weight had a striking action in destroying the parasites of the Indian strain (Adler and Tchernomoretz, 1939), although the parasites of a Mediterranean strain, sometimes referred to as *Leishmania infantum*, were more resistant (Adler and Tchernomoretz, 1941). A Chinese strain in the Chinese hamster *Cricetulus griseus* was also relatively unaffected (Anderson and Soong, 1944).

To the first human patient, an Indian, Adams and Yorke (1939) gave eight daily intravenous injections of 1 mgm. per kgm. of body weight, the total amount of the drug being 360 mgm. After the cessation of treatment the temperature fell to normal, the spleen was reduced in size and the patient was apparently cured. In the case of a woman who had contracted kala-azar in Palestine, Adler and Rachmilewitz (1939) found that stilbamidine led to considerable amelioration, after quinquevalent antimonials had failed. This patient received two intravenous injections of 60 mgm., followed by three intramuscular injections of 100 mgm., and after an interval nineteen intravenous injections of 100 mgm. dissolved in 30 ml. of distilled water. A second Indian male, treated by Adams and Yorke (1940), received a total dose of 400 mgm. The only immediate result was an increase in the daily excursions of the temperature which fluctuated between normal and 104° F. (40° C.). After four days' treatment the temperature began to fall and on the seventh day it reached and remained normal. The spleen, which had extended to the umbilicus, underwent a rapid reduction in size and after six weeks in hospital the patient was discharged, apparently cured. Wingfield (1941) also treated an Indian cook in whom neostam had caused irritation of the skin of the face and eyelids, with œdema. Apparent cure was brought about by ten daily injections of 45 mgm. followed by ten daily injections of 50 mgm.

In the meantime supplies of the aromatic diamidines had been despatched to the Anglo-Egyptian Sudan, where cases of kala-azar had long been recognised as being refractory to treatment (Horgan and Kirk, 1940; Stephenson, 1940), not only because of the frequency of intercurrent infections such as lobar pneumonia and of complications such as cancrum oris, severe diarrhœa, and hæmorrhages, but also because antimony in the form of tartar emetic or the older quinquevalent compounds failed to eradicate the parasites. In their first series of eight Sudanese cases Kirk and Sati (1940a and c) cured six patients who were all in good health six months later: two, who were moribund on admission, died during treatment. The total amount of the drug required to bring about cure varied from case to case. One patient was cured by 975 mgm., given in twenty-four injections over a period of five weeks, whereas another required 4·4 gm., given in seventy injections over a period of six months. For intravenous injections, which were much less painful than those into the muscle, 100 mgm. were dissolved in 10 ml. of distilled water. In a second series of twenty patients two died but eighteen remained in good health for periods of from six to seven months after discharge. The total dosage varied from 0·75 to 4·9 gm., while single intravenous doses varied from 1 to 2·6 mgm.

Two cases which had failed to respond to antimony were cured with stilbamidine, as were two cases with the Sudanese form of muco-cutaneous leishmaniasis. As a rule it was noted that after the first few injections the symptoms increased in severity and skin lesions not infrequently developed, as was the case in patients treated with antimonials (Kirk and Sati, 1940b); the dermal lesions are invariably rich in leishmania. In reviewing their earlier results, Kirk and Sati (1943) found that of twenty-eight cases treated with stilbamidine in the latter half of 1939 and the first half of 1940, four had died in hospital and twenty-four were discharged provisionally cured. One died subsequently, probably not from kala-azar, three were untraceable and twenty were alive and in good health two and a half years later (table, p. 324).

Somers (1944), in the Sudan, also successfully treated five cases with stilbamidine. Injections, which were given every one or three days, varied from 1·1 mgm. to 4 mgm. per kgm. of body weight. Sometimes two courses were necessary at an interval of three or four weeks.

In the Mediterranean area Süsskind and Roth (1943) treated two children aged five and a half and ten years with stilbamidine: the former, who received two courses of the drug, the first consisting of a total of 1·17 gr. in just under three months, the second of 3·75 gm., was in good health two years after completing treatment. The second patient, who had been infected for three years, received the drug dissolved in 10 per cent. glucose; a total of 5·49 gm. was given and the patient was in good health one year after ending treatment. Shellim (1944) successfully cured a patient with two courses of stilbamidine, each of 2 gm., given at an interval of seventeen days. Debono (1947) reported, however, that in small children in Malta stilbamidine had to be abandoned owing to its toxicity.

FINAL RESULTS OF TREATMENT OF FORTY-THREE CASES OF SUDANESE KALA-AZAR WITH AROMATIC DIAMIDINES
(Kirk and Sati, 1943)

	Stilbamidine.	Propamidine.	Pentamidine.	Total.
Admitted to hospital	28	2	13	43
Died in hospital	4	1	3	8
Discharged, provisional cure	24	1	10	35
Died subsequently	1	0	2	3
Alive and good in health after two and a half years	20	1	7	28
Untraceable after two and a half years	3*	0	1†	4

* One case seen and in good health five and a half months after discharge.
† One case seen and in good health 3 months after discharge.

In India, Napier and Sen (1940), and Napier, Sen Gupta and Sen (1942) treated 100 cases of kala-azar with stilbamidine. The patients varied in age from under one year to over forty-five years, and in about half the duration of the disease was at least six months. In ninety-five cases the drug was given intravenously; in five, despite the sharp local reaction, intramuscularly. The usual dosage for early cases was 0·912 ± 0·224 gm. per 100 lb. body weight, or for cases which had resisted antimony 1·009 ± 0·293 gm. Ten or twelve injections were given except in resistant cases, when up to fifteen injections were given. Of the 100 cases two died and two relapsed; one of the relapse cases was treated by a second course.

In the U.S.S.R., Gershenovich and Malaeva (1947) treated nineteen children with stilbamidine with immediate good results in all. Tachycardia was the main unpleasant symptom. The drug was given daily intravenously as a 1·5 per cent. solution. For infants from six to twelve months the first dose was 0·3 ml., increasing daily by 0·1 ml. to 1 ml.; for children from one to three years the first dose was 0·5 ml. increasing daily by 0·2 ml. to 1·5 or 2·5 ml.: older children began with 1 ml. and increased daily by 0·5 ml. to 3 or 6 ml. according to age. From fifteen to fifty injections were considered to be sufficient to bring about clinical cure.

Failures, however, must be recorded. Burchenal and Woods (1945) failed to cure three American soldiers with stilbamidine, and Cole (1944), in East Africa, was unable to cure seven out of fourteen patients. Total amounts of only 1 to 1·3 gm. were given in twelve injections over from fourteen to thirty days, so that the dosage was almost certainly inadequate. Durand *et al.* (1946) failed with two children in Tunis: they were eventually cured with the new antimony compound, 2,168 R.P. Debono (1947) also had failures in children treated with stilbamidine.

Adler and Tchernomoretz (1946) failed to cure dogs infected with kala-azar in the Mediterranean area; even after a combination of 2 gm. of 4 : 4′-diamidino stilbene and 15 gm. of neostibosan residual infection still remained in the skin or at the corneoscleral junction.

Sufficient evidence is now available to show that stilbamidine is a drug of considerable value in kala-azar, more especially in patients who are refractory to antimonials. Children, despite some observations to the contrary, and weak or emaciated adults stand the drug well, and toxic reactions are probably less common than in more robust adults. There is general agreement that the maximum single dose should not exceed 1 mgm. per 1 lb. of body weight. An initial dose of 0·025 gm. per 100 lb. may be increased at the next injection to 0·035 gm. and, provided the reaction is mild, to 0·05 gm. Doses may then be increased by 0·01 or 0·02 gm. to the maximum. Ten injections are usually sufficient for Indian cases which have not shown themselves to be resistant to antimony, but for resistant cases twelve to fifteen injections are more satisfactory; thus varying total dosages of not less than 0·75 gm. per 100 lb. for ordinary cases and 1 gm. for resistant cases are necessary.

Where severe reactions occur adrenalin intravenously may be necessary to restore the immediate fall in blood pressure.

Evidence of satisfactory response is to be found in the fall of temperature and decrease in size of the spleen, together with increased strength and a rise in body weight. After treatment the hæmoglobin should show a 50 per cent. increase, and the total leucocytes should also increase. In their series of 100 cases Napier *et al.* (1942) found that before treatment the total leucocyte count was below 4,000 per c.mm. in seventy-six, whereas after treatment only thirteen patients had counts below this level.

Hydroxy stilbamidine, 4 : 4′-diamidino 2-hydroxystilbene-di-β-hydroxyethane sulphonate, is thought to be less toxic than stilbamidine. Fulton (1944) found that the highest tolerated dose for mice is 1 mgm. and for *Trypanosoma rhodesiense* infections the curative index was 20. In hamsters infected with *L. donovani* it appeared as effective as and not more toxic than stilbamidine. *In vitro* its leishmanicidal activity was 1 in 1,000,000 at 34° C. as compared with 1 in 2,000,000 for stilbamidine (Collier and Lourie, 1946). Sen Gupta (1949a, b) reports having treated six patients in India : five had received no previous treatment and one was partially resistant to antimony. The total dosage was approximately 3 gm. per 100 lb. body weight, given in the course of about twenty injections. One patient had only ten injections, his total dose being 1·39 gm. per 100 lb. body weight. The drug can be given clinically either intravenously or intramuscularly, but the latter route is apt to cause pain. Intravenous injections are well tolerated if given slowly. Immediate clinical cure was achieved in all cases without any marked toxic reaction, but sufficient time has not elapsed to determine the relapse rate.

Pentamidine has been used to a less extent than stilbamidine. Kirk and Macdonald (1940) used this compound on a Sudanese patient who had developed a severe skin eruption following neostibosan. The drug was given intravenously on alternate days in 10 ml. of distilled water. Ten doses of 80 mgm. and thirteen doses of 100 mgm. were given, a total dosage of 2·1 gm. There were no toxic symptoms, the size of the spleen decreased and the cutaneous and nasal lesions subsided. A month later smears from the skin and nose were negative for leishmania.

Later, Kirk and Sati (1940c) treated thirteen patients, three of whom died during treatment ; seven were in good health two and a half years later (Kirk and Sati, 1943). Adams (1941) treated an Indian cook, aged thirty-five years ; daily intravenous injections of 2 mgm. per kgm. of body weight were given for eight days. The temperature fell satisfactorily and the body weight increased despite an alarming but transient fall in blood pressure following one injection.

Napier and Sen Gupta (1943), and Sen Gupta (1944b) treated thirty-two cases in India ; eleven of the patients were refractory to antimony but ten promptly recovered after a course of pentamidine ; of the twenty-one fresh cases nineteen recovered while two failed to respond and were given neostibosan. Two patients, one of them antimony refractory, died of complications. Of twenty-eight patients who recovered six could not be traced, but of the remaining twenty-two, ten were in good health a year later and twelve had relapsed. The dosage employed was the same as for stilbamidine ; the injections were given intravenously. Hazarika (1949) treated fifty-five cases : thirty-four were antimony resistant. Of forty-two followed for six months sixteen relapsed.

In the Mediterranean area Giraud and Revol (1943) cured four of eleven children with intramuscular injections of pentamidine thrice a week. Twelve to fifteen injections were given, 1·5 to 2 mgm. per kgm. of body weight constituting the individual dose. Giraud and Bernard (1946) later recommended 60 to 100 mgm. intramuscularly every other day for twelve to fifteen injections, followed by a second or third course after two to three weeks' interval. In the intervals they gave twelve to fifteen injections of N-methyl glucamine antimoniate (2,168 R.P.) or aminophenyl stibinate of methyl glucamine. Didier (1947) found the action of pentamidine less rapid in reducing fever than that of 2,168 R.P.

The espundia-like condition met with in the Sudan was found by Humphreys (1942) to respond to pentamidine. Two patients, one of whom had relapsed after being treated three years previously with tartar emetic, were apparently cured after receiving a total of 7·7 gm. and 8·5 gm. respectively in sixty-six and seventy-two injections over three months. One patient died later from unknown causes, the other was in good health eight months after ceasing treatment.

In the visceral leishmaniasis of dogs from the Mediterranean zone Faure-Brac (1945) reported that in fifty cases the lesions were rapidly healed by 2 mgm. per kgm. of body weight, given intramuscularly every other day; from fifteen to twenty-five injections were necessary for complete healing, although it is uncertain whether there was complete sterilisation.

Propamidine has been used only to a slight extent. Kirk and Sati (1940c) treated two Sudanese patients with doses of 2 mgm. per kgm. of body weight; one patient died while receiving treatment, the other was in good health two and a half years after the cessation of treatment. Adams (1943) successfully treated an Indian with this compound. Daily injections were given intramuscularly of 100 mgm., or approximately 2 mgm. per kgm. of body weight. For the first four days of treatment the temperature rose after each injection to 104° F. (40° C.); thereafter the daily rise was less marked till by the eleventh day from the beginning of treatment the temperature had fallen to normal. No fall of blood pressure occurred as with stilbamidine or pentamidine, but it was noticed that when two injections were made on successive days into the same region of the deltoid a painful lump formed; a month later it was only slowly decreasing in size. A single injection into the deltoid caused a lump of a week's duration, but deep injections into the buttocks produced no ill effects.

Phenamidine, 4 : 4′ diamidino-diphenyl-ether, has been used by Sen Gupta (1944c and 1945) in India in the treatment of thirty patients with kala-azar; their ages varied from one to fifty years. The drug was given intravenously in 1 per cent. solution for ten consecutive days; this was followed by a second course after a ten-day interval. The initial dose for an adult was 25 mgm., increased daily by 25 mgm. till a dose of 1 mgm. per lb. of body weight was being given. The mean total dose for an ordinary case was 1·73 ± 0·477 gm., or per 100 lb. of body weight 1·854 ± 0·396 gm. Of twenty-four patients who completed the course sixteen were in good health six months later, but of the others, five, who were all that could be traced, had relapsed. No toxic reactions occurred.

Toxicity in Man

Bowesman (1940) in the Gambia, first noted clinically during the treatment of cases of sleeping sickness, that solutions of 4 : 4′-diamidino stilbene (stilbamidine) showed a considerable increase in toxicity. Later, in the Sudan, various toxic symptoms were noted in cases of leishmaniasis treated with the same drug. Concurrently, the activity of the drug was found to diminish and its curative action both in trypanosomiasis and leishmaniasis was reduced.

The conditions bringing about increased toxicity of stilbamidine on standing in aqueous solutions exposed to light have now received considerable attention. Fulton and Yorke (1942) and Fulton (1943) demonstrated experimentally in mice that no change occurred by heating the dihydrochloride at 60° C. for five minutes, by boiling for two minutes, or by keeping at room temperature for fourteen days in the dark. After exposure to sunlight (in Liverpool) for two days the solution of *trans*-4 : 4′-diamidinostilbene (stilbamidine) became yellow and its toxicity for mice increased. The toxicity was not further increased by exposures longer than two hours so that a stable product was presumably formed. The minimal therapeutic dose of the dihydrochloride for a mouse of 20 gm. body weight was reduced from 1 to 0·25 mgm. per kgm., while the therapeutic dose of the di-isethionate was reduced from 2 to 0·25 mgm. Only the unsaturated compounds, diamidinostilbene (R . CH : CH . R, where R is an amidino-benzene group), its monomethyl derivative, and diamidino-tolane are affected in this way.

Barber, Slack and Wien (1943) found that the toxic product differed quantitatively rather than qualitatively from the original substance. They concluded that the chief compound formed was 4 : 4′-diamidinophenylbenzylcarbinol ; this was produced by the addition of water to the ethylenic double bond in the parent substance. As some other therapeutic diamidines do not show such an alteration in biological or chemical properties it follows that the amidine groups are not affected whereas the unsaturated stilbene linkage is involved ; other aromatic diamidines, however, with acetylenic or ethylenic linkages also undergo photochemical changes in aqueous solution (Fulton, 1943). The saturation of this double bond was confirmed spectrographically by Goodwin (1943). Henry (1943) believed that one or both amidine groups were hydrolysed by prolonged exposure to diffuse daylight with accompanying formation of ammonium chloride in the solution. He suggested that in Khartoum the primary products formed were *cis*-stilbamidine, and a dimer of the parent substance, 1 : 2 : 3 : 4-tetra-(4′amidinophenyl)-*cyclo*butane. Fulton and Goodwin (1946), using biological and spectrographic methods, were unable to detect any ammonia formation in solutions exposed to ultra-violet light ; it is therefore concluded that hydrolysis to amide occurs, if at all, only to a negligible degree. When *cis*- and *trans*-stilbamidine are exposed to light the change *cis*→*trans* occurs before the formation of the saturated product of irradiation. No evidence of the reverse *trans*→*cis* change was observed. The formation of the " saturated " product by irradiating aqueous solutions of *trans*-stilbamidine is apparently due to collisions between activated *trans*-molecules.

Fulton and Goodwin (1945b) found that when in the solid dry state 4 : 4′-diamidino-tolane, stilbamidine and 4 : 4′-diamidino-α-methyl-stilbene are exposed to sunlight they often become yellow. In spite of this change there is, however, no significant alteration in their absorption spectra, toxicity for mice or in their therapeutic action as tested against *Trypanosoma rhodesiense* infections in mice. Thus the fact that specimens of stilbamidine have gone yellow after exposure to light is no contra-indication to their therapeutic use. When one of the yellow samples of stilbamidine is dissolved and exposed to light, the same increase in toxicity occurs as in solutions of normal solid specimens under the same conditions.

The nature of the product produced by irradiation of stilbamidine with ultra-violet light has now been elucidated more fully by Fulton and Dunitz (1947), Fulton (1948), and Henry (1948). Many years ago Ciamician and Silber (1902) found that when stilbene in benzene was exposed for a long period to sunlight, a dimer of stilbene was produced with a melting-point of 163° C. The product of irradiation of stilbamidine is also a dimer, 1 : 2 : 3 : 4-tetra-(4′-amidinophenyl)-*cyclo*butane, with a melting-point at 163° C. In addition, a second hydrocarbon with a melting-point at 149° C. is obtained ; this is probably a second isomer which theoretically is obtainable by dimerisation of stilbene.

Examination by Henry (1948) of solutions of stilbamidine which have been stored for three years under various conditions show that the factors upon which the rate of hydrolysis of the amidine groups primarily depends are the *p*H of the solution and the temperature. Exposure to light is not of primary importance but may influence the final result through conversion of the *trans*-stilbamidine to other compounds which may show different susceptibility to hydrolysis and produce hydrolytic products too soluble to be precipitated. A *p*H of 5 suppresses hydrolysis almost indefinitely. Prolonged storage in the body occurs for some eighteen months after a course of treatment with stilbamidine and this compound or some closely related derivative is still being excreted in urine. Pentamidine and the dimer of stilbamidine are both strongly adsorbed by filter paper, and the same conditions in the body of adsorption, storage, and slow release probably apply for the dimer as for stilbamidine. Injection of the dimer in sheep shows that retention of this dimer in the body is very similar to that of *trans*-stilbamidine. From considerations of bond-force constants and bond lengths, decomposition of the dimer would be expected to occur across the longer sides of the rectangle. According to the dimensions of the rectangle given by Fulton and Dunitz (1947), decomposition across the longer sides of the rectangle would involve some 9,000 cal./gram. molecule less than decomposition across the shorter sides and would result in formation of *trans*-stilbamidine and not *cis*-stilbamidine. There is also evidence (Henry 1946) that photochemical decomposition produces *trans*-stilbamidine only and not *cis*-stilbamidine or a mixture of the two isomers. The whole question of the increased toxicity of stilbamidine on exposure to light is still somewhat obscure : *trans*-4-amido-4′-amidinostilbene is rather more toxic than the parent compound and its formation in the body may account for the delayed toxic effects observed. Fulton and Goodwin (1949) have confirmed Henry's observations made in the Sudan. Solutions of stilbamidine maintained at 37° C. for a number of weeks in diffuse light yield 4-carbamyl-4′-amidinostilbene and 4 : 4′-dicarbamylstilbene. The same is true to a lesser extent

HYDROLYSIS OF AMIDINE GROUPS IN SOLUTIONS STORED FOR THREE YEARS
(Henry, 1948)

Compound.	Concentration. %	Conditions.	pH.		Percentage hydrolysed to N_3 compound.	Remarks.
			Initial.	Final.		
Stilbamidine hydrochloride.	1·0	5° C., glass, dark.	6·7	6·50	Nil	Some cryst. of N_4.
,, ,,	1·0	5° C., wax, dark.	6·7	5·98	Nil	Some cryst. of N_4.
,, ,,	1·0	30–40° C., glass, dark.	3·7	5·45	1·7	No visible N_3 cryst.
,, ,,	1·0	30–40° C., glass, dark.	4·5	5·92	5·6	A few N_3 cryst.
,, ,,	1·0	30–40° C., glass, dark.	6·7	6·75	35·5	Heavy crop of N_3 cryst.
,, ,,	1·0	30–40° C., wax, dark.	6·7	5·52	2·6	No visible N_3 cryst.
,, ,, (Soln. init. insolated ninety minutes),	1·0	30–40° C., glass, dark.	6·7	6·45	28·2	Some N_3 cryst.
Stilbamidine hydrochloride. (Soln. init. insolated ninety minutes).	1·0	30–40° C., wax, dark.	6·7	6·05	5·3	No precipitate.
Stilbamidine hydrochloride.	1·0	30–40° C., glass, diffused daylight.	6·7	5·50	6·7	No precipitate.
,, ,,	1·0	30–40° C., wax, diffused daylight.	6·7	5·65	6·3	No precipitate.
Stilbamidine isethionate.	1·5	30–40° C., glass, dark.	—	6·67	26·5	Some deposit of N_2.
,, ,,	1·5	30–40° C., wax, dark.	—	6·25	12·0	Some deposit of N_2.
Pentamidine hydrochloride.	1·0	30–40° C., glass, dark.	7·2	5·65	4·8	No precipitate.
,, ,,	1·0	30–40° C., wax, dark.	7·2	5·15	1·3	No precipitate.

N_4 = *trans*-stilbamidine hydrochloride. N_3 = *trans*-4-amido-4′-amidinostilbene hydrochloride. N_2 = 4, 4′-diamidinostilbene.

when a solution of stilbamidine is kept at the same temperature in the dark. When the same solutions were maintained at temperatures of from 5° to 20° C. the formation of amides did not take place. Good yields of the amides were obtained by autoclaving solutions of the parent substance at one to two atmospheres pressure for several hours.

In man the reactions following injection of stilbamidine have been studied, in India by Sen Gupta (1943) especially, and in the Sudan by Kirk and Henry (1944) and Sati (1949). The toxic effects fall very clearly into two groups, immediate, and delayed. The immediate results consist of a feeling of breathlessness and emptiness in the chest, headache, collapse and faintness, a burning sensation in the veins, formication, sweating, nausea, epigastric pain, vomiting, and occasionally epistaxis. These symptoms are associated with a severe but transient fall in blood pressure. About half of those injected with stilbamidine have slight reactions, about a quarter severe reactions and the remaining quarter slight but unpleasant sensations. Sometimes the reactions are seen after the first or second injection, sometimes not till later. Any fall in systolic pressure below 100 mm. of mercury should be regarded as a reaction.

As a rule reactions pass off in a few minutes, but in a very small percentage of cases the patient may lose consciousness for one to two hours ; there may also be loss of sphincter control. Occasionally a thrombosis may occur in the vein used for injection.

Delayed poisoning comes on in from one to three months with nervous symptoms localised particularly over the area of distribution of the trigeminal nerve. The first symptom usually consists of numbness in the skin areas involved, symmetrical areas over the forehead and cheeks ; later there is paræsthesia, formication, and hyperæsthesia, sometimes accompanied by swelling of the face. Although loss of sensation to light touch is lost, the sense of pressure, temperature and pain is preserved ; in one Indian case the sensation of pain was impaired. Very rarely there may be an extension to the neck, thus showing that lesions are not restricted to the fifth cranial nerve. Sen Gupta believes that the most important lesion is in the principal sensory nucleus of the fifth cranial nerve in the pons.

Collard and Hargreaves (1947) found a high incidence of neuropathy for twenty-two of twenty-four patients treated in Great Britain developed signs and symptoms in from two to eight months after stilbamidine treatment. The first symptoms were numbness or pins and needles over the upper lip and tip of the nose, the patient often noting that he could not feel a cigarette in his mouth. During the next few days or weeks the symptoms spread to the whole trigeminal area on both sides of the mid-line and sometimes to the cervical and dorsal dermatomes. Shaving or a light breeze on the face often caused agonising pain ; itching of the eyelids, with watering of the eyes and sometimes a sticky secretion, together with fine twitching of the muscles round the eyes and frequent blinking were noted. The type of sensory loss was fairly constant, since it consisted of impaired perception of light touch, blunting of pin-pricks, and impaired temperature sensation. Vibration sense was also lost in some cases over the bridge of the nose and the chin. Occasionally, the corneal reflex was depressed. Sensation usually returned in the reverse order to that in which it was lost, but in a few patients where symptoms started over the third division of the trigeminal nerve residual signs persisted in the first division only. Napier (1947) states that trigeminal neuropathy occurs in all cases treated with stilbamidine, usually three to four months after the termination of treatment. One patient became suicidal as a result of hyperæsthesia, formication, and tingling. Hargreaves (1947) also noted trigeminal neuropathy. The nervous symptoms clear up spontaneously but very slowly, for in some instances they have persisted for at least two years. As trichloroethylene inhalations may cause bilateral loss of sensation in the trigeminal area, and in fact have been used to alleviate the symptoms of trigeminal neuralgia, it seems probable that it is the ethylene moiety of the stilbamidine molecule which is responsible for the nervous lesions.

In the Sudan, peripheral neuritis of the legs, with double foot drop, has been reported, and very rarely epilepsy or mania. In addition, fatal delayed poisoning has been observed. A patient who is apparently in good health suddenly develops feelings of nausea, begins to vomit and rapidly passes into a coma which ends in death. Sati (1949) found that of forty-five recovered cases of kala-azar, no less than forty-one still had symptoms of neuropathy, in one case persisting for sixty-seven months after the end of treatment. Although the trigeminal nerve is mainly affected there may be involvement of the seventh and ninth cranial nerves, and of the cervical and upper dorsal nerves. In one patient the development of a conical cornea appeared to be correlated with stilbamidine poisoning. The main signs and symptoms among forty-one Sudanese patients with post-stilbamidine neuropathy were as follows :—

Sign or Symptom.	*No. of cases.*	*Sign or Symptom.*	*No. of cases.*
Itching	39	Twitching of facial muscles	4
Loss of fine touch	31	Ptosis	4
Loss of temperature sense	28	Chest involved	4
Numbness	22	Ulceration of auricles or eyes	4
Blinking	15	Loss of deep touch or pain sensation	4
Neck, anterior part involved	14	Styes	3
Rubbing of eyes	12	Loss of taste and numbness within mouth	3
Neck, posterior part involved	9	Palpitations	3
Formication	9	Twitching of calf muscles	2
Paræsthesia and hyperæsthesia	9	Alopecia	2
Scalp involved	7	Defective vision	2
Loss of tactile localisation	6		
Falling hair or eyebrows	5		

As a rule the signs and symptoms are most marked in those parts of the body exposed to sunlight by the local fashion of dress. This may possibly be correlated with the fact that stilbamidine dimerizes under the influence of light, but at present insufficient is known of the sites of storage of stilbamidine and its breakdown products. Although it usually takes some months for signs of neuropathy to develop, in one patient who was treated with stilbamidine solution which had undergone photochemical changes symptoms of paralysis appeared while the injections were still being given. The signs and symptoms appear to be correlated with the excretion in the urine of a substance giving a blue fluorescence, for of thirty-seven treated cases whose urines were examined thirty-two gave positive results and thirty-one had neuropathy : of six cases who had no signs of neuropathy only one showed a fluorescent substance in the urine. The excretion of the fluorescent substance may continue for years after treatment has ceased.

Among 104 Indian patients treated with stilbamidine Sen Gupta (1943) observed seventeen cases with delayed nervous symptoms, even though the solutions used were fresh. In the Sudan, Kirk and Henry (1944) found that in one series of fourteen patients treated with freshly prepared solutions of stilbamidine seven were cured, four were lost sight of and three died, probably from kala-azar and without nervous symptoms. Of eighteen patients treated with solutions of stilbamidine from one to four weeks old three were cured, five were examined only for one and a half to four months and ten died, the majority with toxic symptoms. Kirk and Sati (1940a) reported two cases in which pentamidine caused alarming epileptiform seizures and unconsciousness after the first and tenth injections respectively. Both patients subsequently recovered. Giraud and Bernard (1946) noted a transient sciatic paralysis in a child following pentamidine. The immediate reactions following propamidine are less than those after stilbamidine or pentamidine and the fall in blood pressure is as a rule small.

The immediate reactions can be prevented or alleviated by administering 0·25 ml. of 1 in 1,000 adrenalin at the same time as the drug, if a reaction has previously occurred after the same dose of diamidine. The tendency to vomit is reduced by giving the injections on an empty stomach.

The effects of calcium in counteracting the fall in blood pressure do not seem to have been examined in man.

For the trigeminal lesions large doses of aneurin appear to be of little value, but Sen Gupta (1943) and Napier (1946) find that intramuscular injections of cobra venom are beneficial; 0·1 ml., rising gradually to 1 ml. of venom, diluted 1 in 100,000, is given every third day. Collard and Hargreaves (1947) did not find that any treatment influenced the progress of the condition, but soluble phenobarbitone gave symptomatic relief to patients with very severe paræsthesiæ.

Reference to the histological lesions produced by the aromatic diamidines was made by Daubney and Hudson (1941), who found extensive fatty degeneration of the liver in cattle given repeated injections of 5 mgm. of pentamidine per kgm. of body weight. Wien *et al.* (1943) reported only slight tubular damage in the kidneys but extensive fatty degeneration of the liver, particularly in guinea-pigs and rabbits and to a smaller degree in mice. With repeated administration of therapeutic doses, however, fatty degeneration in the liver was not observed. In mice and rabbits poisoned with deteriorated stilbamidine Kirk and Henry (1944) noted fatty degeneration of the liver and degeneration of the cells of the convoluted tubules of the kidneys. In patients who had died with symptoms suggesting poisoning by aromatic diamidines, necropsy revealed histological lesions in the liver and kidneys, but it was uncertain how far these changes were actually due to the diamidines. It was also uncertain whether these changes were due to the diamidines themselves or to the toxic substances formed when solutions of stilbamidine are exposed to light. This question has been studied by Oastler and Fidler (1946), whose interest was aroused when two Sudanese patients died while convalescent from kala-azar after receiving 3·15 gm. and 3·05 gm. respectively of stilbamidine over a period of three weeks. The stilbamidine, which was injected intravenously, had been made up as a 1 per cent. aqueous solution and at the time of its administration was from one to four weeks old. One patient died of acute hæmorrhagic pancreatitis three weeks after finishing the course of injections: he had, in addition, minimal central necrosis in the liver lobules and tubular nephritis; the second patient died of hypostatic pneumonia seven weeks after the last injection and at necropsy he was found also to have tubular nephritis, early cirrhosis of the liver, and chronic interstitial nephritis. Dogs were then injected intravenously, some with the stock solution which was now from four to seven months old, and others with freshly prepared stilbamidine. With the stock solution which was given daily the lowest average daily dose was 0·8 mgm., the highest 2·1 mgm. per kgm. of body weight. Of eight animals thus injected only one survived a course of twenty-one injections. The commonest finding was again tubular nephritis and slight necrotic changes in the centre of the liver lobules. A series of ten dogs was then injected intravenously with freshly prepared 1 per cent. aqueous solutions of stilbamidine. The dosage was 1 mgm. per kgm. of body weight during the first week, increasing to 1·5 mgm. and 2 mgm. per kgm. of body weight daily during the second and third weeks respectively. No significant lesions were seen in the liver, kidneys or pancreas, but characteristic pathological changes were noted in the central nervous system in association with clinical signs. Lourie and Yorke (1939) had already reported signs of collapse when stilbamidine was given subcutaneously to puppies in single doses bordering on the lethal but, if the animals recovered from the immediate effects, a peculiar spastic paresis might develop three to four days later. This condition almost invariably ended fatally. Adler and Tchernomoretz (1946) found that many dogs, particularly of the boxer breed, died after a single injection of 1 mgm. per kgm. of body weight given for the treatment of naturally occurring canine leishmaniasis in the Mediterranean zone.

Oastler and Fidler (1946) noted weakness, ataxia, dyspnœa, opisthotonus, head retraction, spastic gait, and loss of consciousness ; in five of the ten dogs treated with the drug these symptoms were associated with swelling of the eyelids and face immediately after the injections. At necropsy, in seven of the animals there were greyish red regions of softening and hæmorrhage in the frontal cortex, both optic thalami, caudate nuclei and throughout the cerebellum and sometimes in the cord. Microscopically the lesions showed hæmorrhage, infiltration with polymorphonuclear leucocytes, and occasional areas of demyelinisation. Perivascular cuffing with small round cells was sometimes seen, while many of the small blood vessels had thickened walls which were infiltrated with polymorphonuclear leucocytes and small round cells. Some of the vessels contained thrombi. Nerve cells showed all stages of degeneration, depending on their proximity to lesions. No relationship appeared to exist between the dosage of the drug and the extent or degree of the lesions.

Oastler and Fidler used solutions which had been autoclaved at 5 lb. pressure for twenty minutes and the lesions which they produced in dogs were, it would seem, probably due to unchanged stilbamidine, Fulton and Goodwin (1949) having shown that solutions of stilbamidine autoclaved under such conditions remain unchanged. Sen Gupta (1947), using freshly prepared or previously heated solutions of stilbamidine, was unable to confirm these findings in monkeys and rabbits given from 2 to 5 mgm. per kgm.

of body weight as single doses or one complete course consisting of fifteen to twenty injections on six days a week. In monkeys there was flushing of the face, at times deep breathing and occasional closing of the eyes and lowering of the head immediately after the injection. During and for three to four months after a course of injections the monkeys remained in good health and on being killed there were no histological changes in the central nervous system. Similarly, in rabbits given rather larger doses of the drug, although there was loss of weight and considerable damage to liver and kidney, with diffuse hæmorrhages in other organs, no histological changes were found in the central nervous system.

In more than 200 patients treated in Calcutta with 1 mgm. per lb. of body weight as the maximum single dose no symptoms such as those described in dogs were ever seen, though two to four months later cases of neuropathy were not uncommon and were seen in patients who had been treated with perfectly fresh solutions.

A peculiar form of delayed poisoning by propamidine in normal cattle has been described by Daubney and Hudson (1941). It followed the subcutaneous injection of doses of 5 to 20 mgm. per kgm. of body weight, usually after an interval of from fourteen to twenty-one days from the last injection. The clinical symptoms were those of complete collapse, generally followed by death in from three to five days. Biochemical changes were a fall in the blood glucose and chloride, a rise in inorganic phosphorus and non-protein nitrogen and the development of a strongly positive van den Bergh reaction. At necropsy there were found œdema of the gastric mucosa with some opaque plaques, and in one instance congestion, mottling, and bile-staining of the liver, petechiæ in the endocardium and epicardium, congestion of the meningeal vessels, and bile staining of the central nervous system. Histologically there was a marked fatty infiltration in the liver cells, which contained only a few glycogen granules, in addition iron storage was subnormal. The tubular cells of the kidney showed cloudy swelling. In cattle given stilbamidine there were no changes apart from a temporary and slight fall in blood pressure.

It seems certain that even freshly prepared solutions of stilbamidine can give rise to lesions in the central nervous system, while fresh solutions in large doses, and solutions which have been exposed to light are toxic for the tubular cells of the kidney, the parenchyma of the liver, and possibly the pancreas. Propamidine is less toxic except in cattle.

The Action *in vitro* of Diamidines on *Leishmania donovani*

A number of early studies on the parasiticidal action of various drugs on *Leishmania donovani in vitro* failed to reach any definite conclusions (Kligler, 1924 ; Noguchi, 1924 ; and Das Gupta and Dikshit, 1929). With the introduction of the aromatic diamidines further studies of drug action *in vitro* were made by Collier and Lourie (1946) and Adler, Tchernomoretz and Ber (1945) ; different methods were employed and somewhat different conclusions were reached.

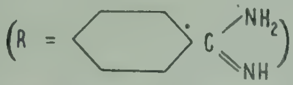
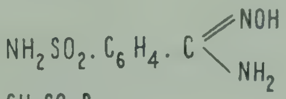
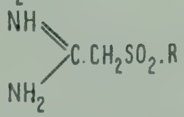
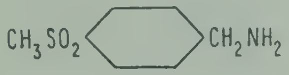
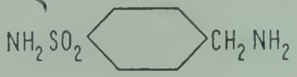
Collier and Lourie (1946) tested the action of diamidines on cultures of *Leishmania donovani*, grown *in vitro* for five days at 34° C., it having been found that the leishmanicidal titre of the drugs was considerably greater at 34° C. than at 24° C., as shown in the table.

ACTIVITY OF AROMATIC DIAMIDINES ON *Leishmania donovani*, *in vitro* AT 24° C. AND 34° C.

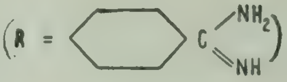
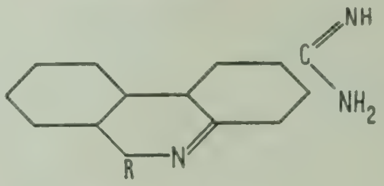
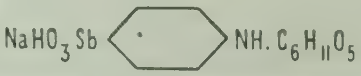
Compound.	Leishmanicidal titre.	
	24° C.	34° C.
4 : 4′-diamidino diphenyl ether (phenamidine)	1 in 50,000	1 in 500,000
4 : 4′-diamidino diphenoxy pentane (pentamidine)	1 in 250,000	1 in 4,000,000
4 : 4′-diamidino stilbene (stilbamidine) . .	1 in 125,000	1 in 2,000,000

The activity of the various compounds tested by Collier and Lourie on *Leishmania* grown *in vitro* and in some cases *in vivo* in the golden hamster, *Mesocricetus auratus*, is shown in the table on p. 341.

THE ACTIVITY OF COMPOUNDS ON *Leishmania donovani in vitro* AT 34° C. AND *in vivo* IN HAMSTERS
(Collier and Lourie, 1946)

Compounds.*		Leishmanicidal titre *in vitro.*	Activity *in vivo* in hamsters.
1. 4 : 4′-Diamidino diphenoxy pentane (pentamidine).	$RO(CH_2)_5OR$	1 in 4,000,000	+
2. 4 : 4′-Diamidino diphenoxy propane (propamidine).	$RO(CH_2)_3OR$	1 in 2,000,000	+ +
3. 4 : 4′-Diamidino-stilbene (stilbamidine).	$R.CH:CH.R$	1 in 2,000,000	+ +
4. 4 : 4′-Diamidino diphenoxy ethane.	$RO(CH_2)_2OR$	1 in 250,000	±
5. 4 : 4′-Diamidino diphenyl ethane.	$R(CH_2)_2R$	1 in 250,000	−
6. 4 : 4′-Diamidino phenyl benzyl ether.	$RCH_2.O.R$	1 in 250,000	−
7. 4 : 4′-Diamidino diphenyl ether (phenamidine).	$R.O.R.$	1 in 250,000	−
8. 4 : 4′-Diamidino dimethyl stilbene.	$R.CCH_3:CCH_3.R.$	1 in 4,000,000	+
9. 4 : 4′-Diamidino diphenyl hexane	$R(CH_2)_6R.$	1 in 2,000,000	−
10. 4 : 4′-Diamidino monomethyl stilbene.	$R.CCH_3:CH.R$	1 in 1,000,000	+
11. 4 : 4′-Diamidino-2-hydroxy stilbene.	$\underset{\underset{OH}{\vert}}{R}.CH:CH.R$	1 in 1,000,000	+ +
2. 4 : 4′-Diamidino tolane	$R.C\vdots C.R$	1 in 500,000	±
3. 4 : 4′-Diamidino diphenyl urea	$R.NH.CO.NH.R$	1 in 500,000	±
4. 4 : 4′-Diamidino diphenyl sulphone.	$R.SO_2.R$	1 in 4,000	−
5. 4-Sulphonamidobenzamidine .	$NH_2SO_2.R.$	<500	−
6. 4-Sulphonamidobenzamidoxime	 $NH_2SO_2.C_6H_4.C\begin{smallmatrix}\diagup NOH\\ \diagdown NH_2\end{smallmatrix}$	<500	−
7. 4-Methylsulphonylbenzamidine	$CH_3SO_2R.$		
8. 4-Amidinomethylsulphonylbenzamidine.	 $\begin{smallmatrix}NH\diagdown\\ NH_2\diagup\end{smallmatrix}C.CH_2SO_2.R$	1 in 500	−
9. 4-Methylsulphonylbenzylamine.		in 8000	−
0. 4-Sulphonamidobenzylamine (marfanil).		<16,000	−
1. 4 : 4′-Diamidino diphenylamine	$R.NH.R.$	1 in 2,000,000	
2. 4 : 4′-Diamidino diphenoxy hexane.	$RO(CH_2)_6OR.$	1 in 2,000,000	
3. 4 : 4′-Diamidino diphenoxy heptane.	$RO(CH_2)_7OR$	1 in 2,000,000	
4. 2 : 2′-Di-iodo-4 : 4′-diamidino diphenoxy propane.	$IRO(CH_2)_4ORI$	1 in 1,000,000	

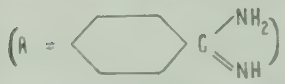
* Diamidines were used in the form of dihydrochlorides, di-isethionates or dilactates. Titres recorded epresent concentrations of the base.

Compounds.*	(R = )	Leishmanicidal titre *in vitro.*	Activity *in vivo* in hamsters.
25. 4 : 4′-Diamidino-αβ-dibromo-dibenzyl.	R . CHBr . CHBr . R	1 in 1,000,000	
26. 2 : 2′-Dibromo-4 : 4′-diamidino diphenoxy propane.	Br . $RO(CH_2)_3OR$. Br	1 in 500,000	
27. 2 : 2′-Dibromo-4 : 4′-diamidino diphenoxy pentane.	Br . $RO(CH_2)_5OR$. Br	1 in 500,000	
28. 2-Bromo-4 : 4′-diamidino diphenoxy pentane.	Br . $RO(CH_2)_5OR$	1 in 500,000	
29. 2-6-Diiodo-4 : 4′-diamidino diphenoxy propane.	$I_2RO(CH_2)_3OR$	1 in 125,000	
30. 4 : 4′-Diamidino dibenzyl ether.	R . CH_2 . O . CH_2 . R	1 in 62,500	
31. 4 : 4′-Diamidino-γδ-diphenyl-hexane.	R . CHEt . CHEt . R	2 in 62,500	
32. 4 : 4′-Di-N-ethylamidinostilbene	EtRCH : CHREt (Et substitutions in the amidino groups.)	1 in 1,000,000	
33 3-Amidino-9-(*p*-amidinophenyl)-phenanthridine.		1 in 500,000	
34. Antimonyl potassium tartrate	$(CHOH.COO)_2K.SbO$	1 in 40,000	
35. N-Glucoside of Sodium *p*-aminophenylstibonate (neostam)		1 in 1,600	
Mepacrine		1 in 400,000	
Proflavine		1 in 400,000	
3349 (Curd, Davey and Rose, 1945)		1 in 100,000	
Surfen C.		<25,000	
Penicillin		Min. lethal conc. 100 units/ml.	

* Diamidines were used in the form of dihydrochlorides, di-isethionates or dilactates. Titres recorded represent concentrations of the base.

The *in vivo* activity of compounds 1 to 7 was tested by Adler and Tchernomoretz (1939 and 1942); of compounds 8 to 20 by Fulton (1944 and 1946).

It will thus be seen that there is some correlation between leishmanicidal activity *in vitro* and chemical constitution, since the most active compounds appear in the following categories



:—

(*a*) $RO(CH_2)_nOR$ and $R(CH_2)_nR$ where *n* is in both groups, more than 2.

(*b*) The α, β-dibromo derivative of the latter group, *n* being 2 as R . CHBr . CHBr . R.

(*c*) R . CH : CH . R and derivatives (2-hydroxy, α-methyl, αβ-dimethyl, and di-N-ethyl).

(*d*) R . NH . R.

Activity of R . $O(CH_2)_nO$. R is generally somewhat impaired by introduction of a halogen into one or both benzene rings. Activity was considerably reduced in the one compound into which two iodine atoms were introduced into one of the rings.

With the exception of (*c*) and (*d*) above, all diamidines with short chain linkages between the aryl nuclei show only moderate or slight activity :—

(*a*) RC : CR and RNH . CO . NHR.

(*b*) RO $(CH_2)_2OR$, $R(CH_2)_2R$, ROR, and RCH_2OR.

(*c*) RCH_2OCH_2R and RCHEt . CHEtR.

(*d*) RSO_2R.

Moderate activity was shown by a phenanthridine compound containing an amidine group in the 3-position and a benzamidine group in the 9-position.

Insignificant activity was shown by sulphonyl and sulphonamido-benzene compounds, containing *p*-amidine, aminomethyl and amidoxime groupings.

These results differ somewhat from those obtained by Adler *et al.* (1945), who came to the conclusion that the degree of correlation shown by diamidines (No. 1 to 7 in the table) *in vitro* and *in vivo* was insufficient for the *in vitro* technique to be regarded as a helpful means of selecting compounds for trial *in vivo*. The technique used by Adler *et al.* (1945) was, however, somewhat unsatisfactory, since the manipulations entailed may well have encouraged chemical changes in certain compounds.

The *in vitro* action of stilbamidine, propamidine and pentamidine on a Sudanese strain of *Leishmania donovani* was examined by Adler *et al.* (1948). The lowest concentration of these compounds which inhibits multiplication does not necessarily interfere with the development of L.D. bodies into flagellates. Stilbamidine is less effective than the other two drugs in both the L.D. body and the flagellate stage, but L.D. bodies are more sensitive than flagellates to all three drugs : propamidine and pentamidine were less effective on a Sudanese than on an Indian strain, but with stilbamidine there was no significant quantitative difference, although Indian kala-azar responds more readily than the Sudanese variety to treatment with this drug. Curiously enough, although pure penicillin has no effect on cultures of leishmania, crude penicillin inhibits its growth.

Ashley *et al.* (1942) and Ewins (1946) compared the trypanocidal activity of aromatic diamidines with chemical constitution but, as shown in the table, trypanocidal and leishmanicidal activities do not run parallel.

RELATIONSHIP OF CHEMICAL CONSTITUTION TO (*a*) TRYPANOCIDAL ACTIVITY *in vivo* AND (*b*) LEISHMANICIDAL ACTIVITY *in vitro* (Ashley *et al.* 1942 and Ewins, 1946)

Chemical category.		Trypanocidal activity *in vivo* (Ashley *et al.*, 1942).	Leishmanicidal activity *in vitro*.
Mononuclear diamidines		Little or none.	Only one compound examined (No. 18); showed negligible activity, though this may be due to SO_2 group in the linkage.
Binuclear diamidines with :	Unsaturated hydrocarbon linkages.	High activity shown by two-carbon linkage RCX : CXR (X = H or CH_3).	High activity shown by two carbon linkage RCX : CXR (X = H or CH_3).
	Saturated hydrocarbon linkages.	Marked activity shown by both two- and six-carbon linkages.	Low activity with two-carbon chain, high activity with six.
Replacement of one CH_2 group by :	O	Slightly enhanced activity.	No evidence of enhanced activity, since titre of RCH_2OR is no higher than that of $R(CH_2)_2R$. Indeed, a suggestion of lowered activity, since titre of $R\,CH_2OCH_2R$ very low, but corresponding $R\,(CH_2)_3R$ not examined.
	NH	Increased activity.	Probably increased activity, since R . NH . R showed high titre, but corresponding R . CH_2 . R was not examined.

Chemical category.	Trypanocidal activity *in vivo* (Ashley *et al.*, 1942).	Leishmanicidal activity *in vitro*.
S or SO_2	Activity practically disappeared.	R . SO_2R inactive.
Chain with one ether linkage.	Homology of this series shows no marked influence on activity. (No compound examined with chain of more than two carbon atoms.)	Inactive or slightly active. R CH_2OCH_2R appreciably less active than R CH_2OR. (No compound examined with chain of more than two carbon atoms.)
Chain with two ether linkages.	Maximum activity with chains of three and five carbon atoms, then diminishing up to ten-carbon chain.	Considerable activity with chains of three to seven carbon atoms. Longer chains not examined.
Replacement of one CH_2 by CH(OH) or by CO.	Diminished activity.	No data.
Substitution in the amidine group.	Substitution of one hydrogen of the amidine group by Me or Et does not seriously impair activity.	Activity not seriously impaired on substitution by Et.
Halogenation of benzene ring or rings (compounds with two ether linkages only).	Diminished activity.	Diminished activity.
Variation in position of the amidine groups.	3′ : 4 diamidines differ little from corresponding 4 : 4′ compounds, but 3 : 3′ compounds less active.	No data.
Monoamidines of similar structure to some active diamidines.	Two amidine groups needed for activity.	No data.
Compounds in which one or both amidine groups are aliphatic in character.	Less active than those with corresponding length of chain in which both amidine groups are aromatic.	

Aromatic Diamidines and Quinquevalent Antimonials

Sufficient time has now elapsed to evaluate the aromatic diamidines and to compare them with the quinquevalent antimonials in the treatment of visceral leishmaniasis. It is now generally agreed that propamidine is inferior to stilbamidine. Pentamidine probably requires further investigation of its therapeutic efficiency; it is certainly less toxic than stilbamidine and less susceptible to decomposition with the formation of toxic degradation products. In the golden hamster Fulton (1944) showed that the leishmanicidal action of 4 : 4′-diamidino-2-hydroxystilbene is quite as high as that of 4 : 4′-diamidino stilbene; doses of 1 mgm. per 20 gm. of body weight three times a week, or a total of ten doses of 20 mgm. of stilbamidine, cured thirteen out of fifteen hamsters, but the hydroxystilbene derivative cured all the animals. The monomethyl and dimethyl derivatives were effective but not as active as stilbamidine, though very active against *Trypanosoma congolense*; 4 : 4′-diamidino tolane and 4 : 4′-diamidino diphenyl urea showed slight activity and the corresponding diphenyl sulphone and diphenyl hexane derivatives were without action. Goodwin and Marshall (1945) found that monoamidines were inactive.

Stilbamidine has now been shown to be active against leishmaniasis in China, India, the Anglo-Egyptian Sudan, and the Mediterranean. In China, Clow (1943) reported that stilbamidine was more rapid in its action than neostam, neostibosan, and urea stibamine.

In India, Napier (1946) believes that stilbamidine is inferior to the best quinquevalent antimonials; it should therefore be reserved for those cases which do not react therapeutically to antimony. For post kala-azar dermal leishmaniasis antimony alone appears to be curative. In Indian kala-azar complicated by tuberculosis stilbamidine eradicated the infection without aggravating the tuberculosis (Sen Gupta, 1944a). In East Africa and the Mediterranean area some failures have been reported with stilbamidine in patients who have subsequently been cured with quinquevalent antimony (Cole, 1944; Burchenal and Woods, 1945; Durand *et al.*, 1946; Debono, 1947), but in some of these cases the dosage of stilbamidine was undoubtedly inadequate.

In naturally occurring canine leishmaniasis stilbamidine is unable, even in combination with neostibosan, to destroy completely the infection, so that treated dogs remain carriers (Adler and Tchernomoretz, 1946).

It is in the Anglo-Egyptian Sudan that the most satisfactory results have been obtained, very largely because of the fact that the local strain of *Leishmania donovani* is so often partially refractory to the older types of antimonial. The lives of many Sudanese patients have undoubtedly been saved by stilbamidine. It is, however, essential that the solution of stilbamidine should be freshly prepared and that it should not be exposed to bright light before injection. The more recently introduced sodium antimony gluconate is undoubtedly preferable.

In stilbamidine, however, there is available a compound which can be used as an alternative to antimony and, unlike the older antimonials, is capable of curing human visceral leishmaniasis in many parts of the world, since in varying degrees all strains of *Leishmania donovani* appear to be susceptible to its chemotherapeutic action (Kirk, 1944). The main drawbacks to its use lie in the possibility of alarming but not serious immediate reactions which can largely be prevented, and in the occurrence in a high percentage of persons of very serious nervous sequelæ.

Before the introduction of antimony the death rate from kala-azar in India was very high. With the use even of tartar emetic it was successfully reduced to 88 per cent. in the Sibsagar epidemic of 1921 (Rogers, 1939), while in 1942 Napier, Sen Gupta and Sen (1942) were able to report a mortality rate of under 2 per cent. In the Sudan the mortality rate is still about 25 per cent. (Kirk, 1947); this may be due to differences in the genetic constitution of the host or more probably to differences in the parasites, Chinese and Indian strains of kala-azar reacting more readily than Sudanese and Mediterranean strains.

It may here be emphasised that in addition to specific treatment, general and symptomatic measures are essential if either antimony or the aromatic diamidines are to exercise their full curative action. Intercurrent infections must be eliminated, anæmia counteracted, and nutritional deficiencies made good. Prolonged observation after apparent cure is also essential.

Penicillin, while it has no direct effect on leishmania, appears to be of assistance, especially in children, when there are signs of broncho-pneumonia or if cancrum oris develops.

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CUTANEOUS AND MUCO-CUTANEOUS LEISHMANIASIS

Under this heading are included (1) oriental sore, due to *Leishmania tropica ;* (2) the muco-cutaneous form of leishmaniasis, espundia, found in South America, and the very similar condition occasionally met within the Anglo-Egyptian Sudan ; and (3) dermal leishmaniasis, first described in India by Brahmachari (1922), in association with visceral leishmaniasis, and now known to follow therapy not only with antimonials but also with the aromatic diamidines.

For the last condition the diamidines appear to be quite ineffective and quinquevalent antimony is the only treatment. In some cases the amount of quinquevalent antimony necessary to bring about a cure is large.

Oriental Sore

After the introduction of tartar emetic in South American leishmaniasis by Vianna (1912), antimonyl tartrates were naturally employed in the treatment of oriental sore, but intravenous injections of tartar emetic are now regarded as a somewhat drastic procedure for a disease usually without serious consequences. The difficulty of determining the value of any particular drug in the treatment of oriental sore lies in the fact that the disease tends to spontaneous healing; Dostrowsky (1929), for instance, found that spontaneous cure might occur after three to seventeen months.

Few critical attempts have been made to determine the exact value of the many drugs which at one time or another have been acclaimed as having a curative action.

Intensive experiments have been made with berberine acid sulphate (orisol) (Chopra *et al.*, 1932). The alkaloid berberine, isolated in 1826, occurs in many species of *Berberis* and is usually either extracted from bark of the barberry, *Berberis vulgaris*, or obtained as a by-product in the manufacture of hydrastine from the Golden Seal, *Hydrastis canadensis.* A crude extract made from berberis plants has long been used in Indian indigenous medicine under the name of "rasant," and as long ago as 1911 Jolly employed rasant in the treatment of oriental sore. The alkaloid berberine was first used by Varma (1927), and later by Karamchandani (1927) and others. Hayward (1933) found that all cases which received five or more injections of a 2 per cent. solution of berberine acid sulphate were cured, but the injections caused so much pain that many patients refused to continue treatment.

Ball and Ryan (1944) reported that of 138 cases treated by the infiltration of 1 ml. of berberine acid sulphate, 31·8 per cent. were failures: they, however, used a 1 per cent. instead of the more usual 2 per cent. solution of "orisol." Das Gupta and Dikshit (1929) claim that berberine sulphate has a direct action *in vitro* on both *Leishmania tropica* and *L. donovani.*

Gupta and Kahali (1944) isolated an alkaloid umbellatine, $C_{21}H_{21}O_8N$, from *Berberis umbellata* and *B. insignis.* This alkaloid behaves very much as does berberine and in controlled tests appears to be as effective as berberine acid sulphate in treating unbroken oriental sores. In a dilution of 1 in 50,000 umbellatine is said to inhibit the growth of *Leishmania tropica* in a liquid hæmoglobin-saline medium. Even in a dilution of 1 in 10,000 it had no inhibitory action on *L. donovani*, grown in the same medium.

More recently mepacrine has been used to infiltrate the lesions.

This method was originally introduced by Flarer (1938) in Catania.

Mepacrine has been employed particularly by workers such as Marchionini (1941) in Italy, and Cupi and Cattapan (1942) in Ankara and Asmara. The sores are infiltrated intradermally by means of a dental syringe with 1 to 4 ml. of a 10 or 20 per cent. solution of mepacrine, according to the size of the sore; this process is repeated once or twice at intervals of eight to ten days. The actual injections are somewhat painful when, as is so frequently the case, multiple sores are present. Marchionini gives in addition intramuscular injections of mepacrine on alternate days; the first injection is 0·1 gm., the second 0·2 gm., while 0·3 gm. is given on the fifth, seventh and ninth days. In early cases healing is rapid and there is very little scarring; in old-standing cases the results are not so good.

These results have been confirmed by Sachdeva (1943) and Elkerton (1944) in India. Mepacrine injections containing 0·05 gm. per ml. were employed every three to five days. Healing occurred in from one to ten weeks, half the cases being cured within three weeks. The rate of healing was no more rapid than with a routine treatment such as scraping the sore under a general anæsthetic and a weekly dressing of tannic acid powder; mepacrine, however, was simpler and scarring was minimal. In sores with much ulceration the injection of mepacrine may cause considerable local reaction (Mechin, 1946).

In the U.S.S.R. Dobrotvorskaya (1947) used a 5 per cent. solution of mepacrine for sores not older than three months: it is advisable to allow at least one lesion to run a natural course in order to develop a natural immunity. Lesions of from four to five months' duration are more apt to relapse. With older lesions a 2 per cent. solution completely cured 104 sores, retarded the development of 101, but failed completely in one.

It has long been recognised that quinquevalent antimonials will eventually cure oriental sore, but it may be doubted whether the prolonged use of these drugs for a disease which undergoes spontaneous healing is entirely justified.

Ball and Ryan (1944), for instance, found that of 208 European patients, infected probably in Persia, all were eventually cured by weekly intravenous injections of neostam: however, the average time required for healing was 14·5 weeks, while the total amount of drug necessary to effect a cure averaged 1·14 gm. If single injections exceeded 0·15 gm. they caused unpleasant reactions in a high percentage of cases.

Berberian (1945) believed that intravenous injections of neostibosan hastened the healing of sores in the ulcerating stage, whereas weekly local injections of 1 to 2 ml. of a 10 per cent. solution of mepacrine had no effect.

Vilanova (1943) used concentrated solutions of sodium antimony gluconate locally in the treatment of oriental sore. The dose employed was 0·4 ml. per 10 kgm. of body weight, but not more than 2 ml. should ever be given at any one time. For sores on the eyelids, nose, and ears this appears to be a method of value, for parasites disappear from the lesions within twenty-four hours. Kikuth and Schmidt (1943) found that sodium antimony gluconate was the only antimonial which, after twelve local injections, removed all leishmania from experimental oriental sores on the mouse tail. As this quinquevalent antimonial is of low toxicity further investigation of its action on oriental sore is desirable.

The correct evaluation of chemotherapeutic remedies in oriental sore is, as previously mentioned, notoriously difficult, for not only does the infection tend to undergo spontaneous cure, but the success of any drug depends on the age and character of the lesions ; in addition there is growing evidence that there exist different strains of *Leishmania tropica*, some of which may be more susceptible than others to chemotherapeutic drugs.

Muco-cutaneous Leishmaniasis

If the treatment of oriental sore is still uncertain, that of muco-cutaneous leishmaniasis is even more unsatisfactory, since for reasons which are at present unknown the muco-cutaneous lesions of South American leishmaniasis are much more resistant to antimony than infections due to *Leishmania donovani*. This is especially true of the mucous lesions which develop sooner or later in untreated cases. These mucous membrane lesions yield to antimony far less readily than those of the skin (Carri, 1943).

In a few cases where cutaneous lesions alone are present, potassium permanganate, berberine, tartar emetic, and stibophen

(Kean, 1944) have brought about a cure. Peña Chavarra *et al.* (1943) claim successful treatment of American cutaneous leishmaniasis by oral administration of a solution of tartar emetic, 2 gm. of the drug being dissolved in 80 ml. of chloroform water so that 1 mgm. of tartar emetic was contained in one drop of the solution. In children, treatment was begun by giving a drop a day and increasing the dose by three or four drops a day till the limit of tolerance was reached : in adults the initial dose was five to fifteen drops and the dose was again increased to the limit of tolerance. Complete healing occurred in from three to four months.

Mazza and Cornejo (1940) used mepacrine in South American leishmaniasis. Infiltration of the cutaneous lesions was carried out with 5 ml. of a 10 per cent. solution of mepacrine while 0·3 gm. was taken internally for seven days. The lesions in three cases healed in a week.

In early South American muco-cutaneous leishmaniasis melarsen oxide is of value if given intravenously six or seven times daily. Healing is completed by the twentieth day. More advanced cases were found by Fernandes and Payne (1946) to be resistant to melarsen oxide but susceptible to injections of a tervalent antimonial compound of methyl-hydroxy-propylaminethoxy-catechol in an aqueous solution of *iso*propylamine containing 8·5 mgm. of metallic antimony per ml. : five to nine doses of 2 ml. every second day completely healed the lesions by the twentieth day.

The oral leishmaniasis occurring in the Sudan responds to intravenous injections of tartar emetic or neostibosan (Humphreys and Mayne, 1935).

Antibiotics. Tyrocidine in concentrations of 5·0 gm. per ml. markedly inhibited the growth of *Leishmania tropica* in cultures (Weinman, 1943), but penicillin was without effect either on cultures of *L. tropica* or in experimentally infected hamsters (Fulton, 1945). Snow (1944) failed to cure a case of cutaneous leishmaniasis involving the nose by local application of 50,000 units of penicillin, combined with the intramuscular injection of 559,000 units, and Horgan and Sati (1945) observed no benefit from the treatment of two Sudanese cases of kala-azar with in each case a total of 1,500,000 units of penicillin.

The drug is of value, however, in clearing up broncho-pneumonia and cancrum oris, which are apt to be fatal complications in leishmaniasis of children.

There is no evidence that the sulphonamides have any action on *Leishmania*. In some cases where cutaneous leishmanial lesions have become secondarily infected with bacteria, sulphonamides or penicillin have been of value in removing these bacteria.

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CHAPTER VII

THE CHEMOTHERAPY OF TRYPANOSOMIASIS

Although much of the earliest experimental work in chemotherapy was carried out on trypanosomes, trypanosomiasis of man and of domestic animals is still a major problem in many parts of Africa, while in South America the infection caused by *Trypanosoma cruzi* is still largely unaffected by chemotherapeutic drugs.

Trypanocidal action, so far as it can be judged from the effects on trypanosome infections in small animals, is a property possessed by a wide variety of chemical compounds, metallic and non-metallic, with few obvious chemical or physical properties in common. Nevertheless the number of compounds actually used in the treatment of trypanosomiasis of man and of domestic animals is comparatively small. To the well-established quinquevalent arsenicals must now be added melarsen, melarsen oxide and *p*-arsenophenylbutyric acid (butarsen), and among the non-metallic compounds, in addition to suramin, the aromatic diamidines, more especially pentamidine, seem likely to play an increasingly important *rôle*, especially in the chemoprophylaxis of the disease in man. In the chemotherapy of trypanosomiasis of domestic animals phenanthridinium compounds and antrycide are of particular interest.

METAL-CONTAINING COMPOUNDS

The metals which have been found to be of particular value in trypanosomiasis are arsenic and antimony.

Arsenical Preparations

(1) **Tryparsamide** (tryparsone, tryponarsyl, tryparsonum, novatoxyl, trypotan, glyphenarsinum) is sodium N-phenylglycine-amide-*p*-arsonate and should contain not less than 25·1 per cent., and not more than 25·5 per cent. of arsenic in organic combination, together with not less than 9·25 per cent. and not more than 9·5 per

cent. of nitrogen, both figures being calculated with reference to the substance dried at 110° C. Tryparsamide is soluble to the extent of 3 parts in 10 of water and forms a neutral solution: it is almost insoluble in 95 per cent. alcohol, chloroform, ether, and benzene. In giving injections the drug is dissolved in glass-distilled sterile water or sterile physiological saline: toxic reactions are more likely to occur if boiled and filtered water is employed. No biological test of toxicity is officially prescribed in Great Britain, but the following method for estimating toxicity is usually recommended:—

Five well-nourished adult male rabbits are injected intravenously with a dose of 0·75 gm. of tryparsamide per kgm. of body weight in a 10 per cent. aqueous solution. Three of the five animals should survive without signs of serious intoxication. The period for which the animals are observed is seven days, but Launoy and Prieur (1935) prefer twenty days. Pottier and van den Branden (1935) suggest 1·0 gm. in place of 0·75 gm. per kgm. of body weight as the test dose, but this modification has not been generally adopted. A further test for toxicity is recommended by van den Branden and Pottier (1938), in which ten white mice of 30 gm. weight are injected subcutaneously with 0·09 gm. of tryparsamide dissolved in ten times its weight of distilled water at a temperature of 20° C. Not more than four of the ten should develop nervous symptoms and at least six should survive for ten days.

Further biological tests are desirable to determine whether as a result of storage tryparsamide has developed any undue tendency to give rise to optic neuritis.

Fulton and Yorke (1943) showed that the maximum tolerated dose of tryparsamide for mice varies according to the route of administration, the amount tolerated orally being twice that tolerated by other routes. The toxicity for mice by different routes and the therapeutic efficiency of tryparsamide against *T. rhodesiense* are shown in the table on page 361.

In the case of reduced tryparsamide there are considerable differences in the amounts tolerated by the different routes: the values for the M.E.D. and M.C.D. are exactly the same following subcutaneous, intraperitoneal, and intravenous admini-

Toxicity for Mice of Tryparsamide and Therapeutic Action by Different Routes against *T. rhodesiense* (mgm. per 20 gm. Mouse). (Fulton and Yorke, 1943.)

Route.	M.T.D.	M.E.D.	M.C.D.	$\frac{\text{M.T.D.}}{\text{M.E.D.}}$	$\frac{\text{M.T.D.}}{\text{M.C.D.}}$
Oral . .	120	20	80	6	1·5
Subcutaneous .	60	10	20	6	3
Intraperitoneal .	60	10	20	6	3
Intravenous .	60	10	20	6	3

M.T.D. = Maximum tolerated dose.
M.E.D. = Minimum effective dose.
M.C.D. = Minimum curative dose.

stration: consequently the M.T.D. governs the values of the therapeutic indices obtained. The results are shown in the table:—

Toxicity for Mice of Reduced Tryparsamide and Therapeutic Action against *T. rhodesiense* by Different Routes (mgm. per 20 gm. Mouse). (Fulton and Yorke, 1943.)

Route.	M.T.D.	M.E.D.	M.C.D.	$\frac{\text{M.T.D.}}{\text{M.E.D.}}$	$\frac{\text{M.T.D.}}{\text{M.C.D.}}$
Oral . .	8	1	2	8	4
Subcutaneous .	2	0·05	0·1	40	20
Intraperitoneal .	2	0·05	0·1	40	20
Intravenous .	0·5	0·05	0·1	10	5

M.T.D. = Maximum tolerated dose.
M.E.D. = Minimum effective dose.
M.C.D. = Minimum curative dose.

In the case of reduced tryparsamide at least, the trypanocidal effect of a relatively high concentration of drug in the blood for a short period is about the same as that for a relatively low concentration over a longer period.

Reduced tryparsamide acts against *T. rhodesiense* more rapidly than tryparsamide itself, and *in vitro* the minimal trypanocidal concentration is 1 : 200,000, whereas the corresponding figure for tryparsamide itself is 1 : 1600.

The Blood-brain Barrier in Trypanosomiasis. Tryparsamide owes its great value in sleeping sickness not to its trypanocidal action, which is low, but to its curative action in the secondary stage. This action of tryparsamide is due to the fact that it penetrates the barrier between the blood and the brain with greater facility than suramin, neocryl or aromatic diamidines; although in rabbits tryparsamide is therapeutically inferior to neocryl and pentamidine (Lourie and Yorke, 1939), yet in later infections in man it is superior (Harding, 1940; Bowesman, 1940; Lourie, 1942).

The capacity of tryparsamide to affect trypanosomes in the central nervous system involves a brief consideration of the blood-brain barrier in man. It is now generally agreed that the barrier in man is constituted, not by the choroid plexus or the meningeal vessels, but by the walls of the cerebral capillaries (Broman, 1941; Friedemann, 1942). In man these capillaries are surrounded by a layer of glial cells. As Lourie (1943) has pointed out, methods of testing for trypanocidal action after passage of a drug through the blood-brain barrier are not very satisfactory and further studies are required. The trypanocidal power of the cerebrospinal fluid after administration of various arsenicals was originally studied by Voegtlin *et al.* (1923); later investigations were made by Hawking *et al.* (1937, 1938) and Hawking (1940). Sulpharsphenamine, neoarsphenamine, tryparsamide, and orsanine render the cerebrospinal fluid trypanocidal, but neocryl and reduced thioglycollate do not. Among non-polar substances the factors which allow a compound to appear in the cerebrospinal fluid include lipid solubility and surface activity, but with polar substances molecular size, lipid solubility and diffusibility are minor factors. As was originally shown by Wittgenstein and Krebs (1926), the electric charge is all important, those substances which are negatively charged passing through readily whereas positively charged compounds do not pass. Other factors favouring penetration are diffusibility and degree of dispersion: substances of a colloid nature do not appear in the cerebrospinal fluid. It is possible that in trypanosomiasis of the central nervous system the permeability of the blood-brain barrier may be increased. Such is known to be the case in general paralysis of the insane (Könyves-Kolonics and Huszak, 1948). It must be emphasised that most studies have dealt with the blood-cerebrospinal fluid barrier which is quite distinct from the true blood-brain barrier.

It is, in fact, unjustifiable to assume that because a compound is present in the cerebrospinal fluid it must have passed through the blood-brain barrier. The cerebrospinal fluid is formed mainly by the choroid plexus in the lateral ventricles ; thence it passes through the foramina of Magendie and Luschka into the subarachnoid space, whence it is absorbed into the blood stream by way of the arachnoid villi in the cranial venous sinuses. This circuit does not traverse the blood-brain barrier. Although the bulk of the fluid is undoubtedly produced by the choroid plexus, a small amount may be due to fluid reaching the subarachnoid space directly from the perivascular spaces of the brain (Weed, 1922). Thus, on the one hand a compound found in the cerebrospinal fluid may not all have traversed the blood-brain barrier : on the other hand, failure to detect a trypanocidal compound in the cerebrospinal fluid may be due to the fact that though it may have been present in effective concentrations in the minute perivascular spaces, it has been diluted on reaching the subarachnoid space.

If the central nervous system is to be freed from trypanosomes it is necessary for the trypanocidal drug to reach the parasites not only in the cerebrospinal fluid but in the brain tissue, perivascular spaces, and perivascular cellular infiltrations, as well as those parasites which are actually in the walls of small blood vessels and capillaries.

Unfortunately there is no known method by which the blood-brain barrier may invariably be rendered increasingly permeable. Weed and Hughson (1921), however, showed that if a 30 per cent. solution of sodium chloride is injected intravenously the pressure of the cerebrospinal fluid after a sharp rise falls profoundly for two to four hours. This has the effect of reversing the normal flow of fluid in the perivascular spaces which is normally towards the surface of the brain (Weed and McKibbon, 1919 ; Foley, 1923).

No data are yet available to show whether in advanced sleeping sickness hypertonic sodium chloride given intravenously is of practical value by increasing the amount of tryparsamide in the cerebro-spinal fluid.

The claim made by Lieurade (1934), van den Branden and Pottier (1934), and van den Branden and Appelmans (1935) that hexamethylene tetramine increases the permeability of the blood-brain barrier to tryparsamide has not been confirmed : it undoubtedly irritates the kidneys. A full discussion of the various questions involved in the penetrations of substances into the cerebrospinal fluid and brain is given by Merritt and Fremont Smith (1937), and Broman (1949).

Treatment by Tryparsamide. Tryparsamide has occasionally been used alone in the treatment of sleeping sickness in the primary stage, but as a rule it is now combined with suramin. In the opinion of French workers it is " le moins bon des trypanocides." Chesterman (1932), however, treated first-stage cases with tryparsamide. For children the dose should be 0·07 gm. per kgm. of body weight, for young adults 0·055 gm. per kgm. of body weight, and for adults 0·045 gm. per kgm. of body weight. Six to eight weekly injections are given. Second courses are not recommended. In second-stage cases the same number of injections is given but the doses are larger, 0·09 gm. for children, 0·07 gm. for young adults and 0·06 gm. per kgm. of body weight for adults. In advanced second-stage cases two or three large doses should be given at five days' interval followed by a rest of ten to fourteen days. Relapses are often associated with some degree of resistance to tryparsamide. Loré and Marty (1933) found that patients who, despite having received a course of tryparsamide, pass into the second stage are liable to die. On the other hand, patients in the nervous stage who are then first given tryparsamide, even though they have previously received atoxyl, have a good chance of recovery. This curious fact has been noted also by Chesterman (1932), but Millous and Maury (1934) and Bonnet (1934) do not agree that tryparsamide should be reserved for second-stage cases.

The percentage of cures produced by tryparsamide alone has varied greatly. Chesterman (1932) believed that 50 per cent. of second-stage patients infected by *T. gambiense* may be saved but this may entail a considerable percentage of blindness. Kellersberger (1933, 1936) reported remarkably good results after fifteen weekly injections, the course being repeated after a few months. Lester (1932) found that tryparsamide has a remarkable effect in reducing the cell count in pathological cerebrospinal fluids: in fifteen out of eighteen cases the count had returned to normal after the second injection of tryparsamide. Laurent (1932) observed normal cell counts in 148 of 166 cases after treatment with tryparsamide, but it is to be noted that a raised cell content a few weeks after treatment is not an indication of failure, since the cell content may take some time to return to normal. On the other hand, tryparsamide may on occasion induce a lymphocytosis in a normal cerebrospinal fluid (Barlovatz, 1933a) or, more rarely. give rise to a Jarisch-Herxheimer reaction in the central nervous system (Mackie, 1935).

In cases with nervous involvement the first few injections not infrequently cause an exacerbation of the mental symptoms but in late second-stage cases tryparsamide often has a remarkable tonic effect, sleepiness and headache being abolished and the patient kept alive for months or years. Some patients have even returned to work. There is, however, no improvement as judged by the condition of the cerebrospinal fluid.

Reduced Tryparsamide. Whereas tryparsamide has been and still is of very considerable value for attacking trypanosomes in the central nervous system, it has a low trypanocidal action on the parasites in the blood, largely because it is eliminated so rapidly that only a comparatively small part is reduced to the tervalent form. According to Launoy and Fleury (1937), from 88 to 95 per cent of a given dose injected intravenously is eliminated within the first hour by way of the kidneys. Tryparsamide acted on by thioglycollic acid, " reduced " tryparsamide, has a high direct trypanocidal action but apparently a considerable degree of chemical instability and a high toxicity. These disadvantages have been overcome by Friedheim (1949a), who by methods which are undisclosed claims to have produced a reduced tryparsamide of low toxicity and considerable stability. " TPB " is a white powder containing 21·6 per cent. of tervalent arsenic: it can be given to man either by mouth or intravenously. By mouth a dose of 10 mgm. per kgm. of body weight can be given daily for eight days: after an interval of a week the course can be repeated. Intravenously, 1·5 mgm. per kgm. of body weight can be given daily for five days, followed by a rest for a week; thereafter a second similar course can be administered. A single oral dose of 7 to 25 mgm. per kgm. of body weight removes trypanosomes from the blood in twelve hours and from the lymph nodes in twenty-four hours. A single intravenous injection of 1·5 mgm. per kgm. removed trypanosomes from the lymph nodes in from one to six hours. Ten previously untreated cases of second-stage sleeping sickness were treated with from seven to fourteen oral doses of from 7 to 25 mgm. per kgm. of body weight. Trypanosomes promptly disappeared from the cerebrospinal fluid, and the number of cells fell rapidly as did the protein content. TPB had no effect either on a tryparsamide-resistant strain of *T. gambiense* in the guinea pig or on one human second-stage case harbouring a resistant strain of the same species of trypanosome.

In view of the good results obtained in the intensive arsenical therapy of syphilis, an attempt has been made to see whether it is possible to employ intensive tryparsamide therapy. Fowler (1945 and 1947) gave tryparsamide by an intravenous drip method to seventy-two patients with Gambian sleeping sickness, fifty-six of whom gave evidence of involvement of the central nervous system. The dose was 2 gm. daily dissolved in 2 pints of sterile double-distilled water: it was given for six to nine days, though in some patients a few days' rest was necessary because of the febrile reactions.

The daily dose was given over a period of eight hours daily, the rate being approximately forty drops a minute. All the patients had high fever and in five there was jaundice: eight had impaired vision and eleven died. In two patients the sight failed to improve after the termination of therapy. The treatment caused less disturbance in children than in adults. Clinically most of those patients who survived were strikingly improved. The posterior cervical lymph nodes decreased in size, somnolence was lost and mental acuity increased. A further examination of thirty-nine cases in from six to twenty-four months showed that thirty-two had apparently recovered, four had improved and three showed no change. The use of a drip for eight hours daily is, however, inconvenient, especially in understaffed hospitals. Fowler (1947) therefore tried the effect of a daily injection of 10 ml. containing 2 gm. of tryparsamide until from 14 to 16 gm. had been given. Of eleven adults with nervous symptoms all showed clinical improvement and only one had optic neuritis. Unfortunately six months later only three patients could be examined but all were cured. The cell content not infrequently rose immediately after treatment, to return to normal later.

Toxic Reactions due to Tryparsamide. Apart from occasional cases of erythema and very rarely of exfoliative dermatitis, the only toxic reactions usually encountered are those associated with optic atrophy. Lourie (1942), however, in dealing with people from the Kissi tribe in Sierra Leone whose diet at the time was quite inadequate, noted that tryparsamide caused cough, diarrhœa, fever, papular or maculo-papular rashes, itching, sore throat, œdema, lachrymation, epididymitis and orchitis, septic sores on the soles of the feet and, in one instance, facial palsy. It has been recognised for many years, in fact since atoxyl was first used in treatment, that certain organic arsenicals are likely to cause optic atrophy. From animal experiments, Young and Loevenhart (1924) showed that in organic compounds an amino- or substituted amino-group in the *para*-position was prone to cause optic lesions. The fact that optic changes have occurred when tryparsamide has been used in the treatment of syphilis (Woods and Moore, 1924) rules out the possibility that the condition is due to the toxins of dead trypanosomes. The incidence of optic changes is not always easy to determine in the absence of an ophthalmological specialist since trypanosomiasis may itself give rise to ocular changes (Scott, 1944 ; Ridley, 1945), especially in under-nourished African populations. Habig (1949) describes acute inflammatory changes in the central artery. The incidence of optic changes due to tryparsamide varies very considerably as is seen from the table on p. 368. Some observers have adopted a very rigorous standard in assessing these changes.

Saunders (1944), who has had an enormous experience in the Gold Coast, believes that the incidence is less than 1 per cent., but Lourie (1942) finds that the percentage depends on the treatment used and on the duration of infection. In very late cases given suramin and tryparsamide nine of seventy-nine cases were affected. After nine or ten injections the incidence was 4·9 per cent., but after five to seven injections only 0·8 to 0·9 per cent. In certain series, however, as in West African soldiers examined by Ridley (1945), it seems probable that toxic changes had occurred in the drug after two and a half years' sojourn in the tropics, although the ordinary tests did not indicate any increase in toxicity. A rose colour, however, develops in solutions made from drugs which have been kept in store for a prolonged period. Van den Branden and Dumont (1933) regard this colour reaction as due to impurities and not to the drug itself. When tryparsamide was incubated for three months in a saturated atmosphere it became yellow and more toxic though no change could be detected in its physical or chemical properties. Van Hoof (1933) suggested that suramin aggravated the effect of tryparsamide by irritating the meninges, while van den Branden and Appelmans (1935) believe that trypanosomiasis may damage the optic nerve and render it more susceptible to tryparsamide, the "terrain trypanosomé": a raised pressure in the cerebrospinal fluid may also be an accessory factor.

INCIDENCE OF OPTIC LESIONS FOLLOWING TRYPARSAMIDE

Number of cases of sleeping sickness.	Number with optic atrophy.	Percentage.	Evanescent symptoms.	Observer.
25,638	323	1·23	—	Jamot (1929).
118	24	20·3	—	Van den Branden and Appelmans (1934).
8,393	8	0·01	13 (0·02 per cent.).	De Brauwere (1938a and b).
132	2	1·49	—	Ridley (1945).
39 (West African soldiers)	9	23·0	—	Ridley (1945).
152	1	0·65	—	Scott (1944).
77	9	11·7	—	Pearce (1921).
241	13	5·5	25 (10·3 per cent.).	Woods and Moore (1924).
460	16	3·5	—	Sloan and Woods (1936).
500	4	0·8	—	Potter (1943).
Total 35,750	409	1·1		

There is some evidence that nutritional deficiencies may predispose to optic atrophy, more especially lack of vitamins A and the B group. McDermott *et al.* (1943) showed that whereas rats deprived of either vitamin A or the vitamin B complex were more liable than normal animals to optic atrophy following tryparsamide some of of the controls subject only to lack of vitamins developed a very similar condition. In the case of African soldiers, large doses of yeast seemed to make little or no difference to the rate of recovery.

Sloan and Woods (1936) distinguish two types of objective reaction due to tryparsamide: the acute type, which is rare, is characterised by rapid loss of vision followed by almost complete blindness: the chronic reaction is characterised by contraction of the visual fields with retention of normal central vision and absence of objective signs of atrophy of the optic nerves.

In the African in whom the majority of cases of optic atrophy due to tryparsamide have been recorded, four stages may be distinguished. First, the condition is purely subjective, the patient complaining of metamorphopsia and a sensation of shimmering movements. Africans often describe this as though they were looking through waving smoke and illustrate the sensation by making fluttering movements with the outstretched fingers. Secondly, within a few days, objective phenomena appear for there is a general depression of vision with loss of the peripheral field even for large objects, followed by decrease in central acuity. The appearance of the optic discs still remains normal.

In all probability peripheral field loss to small objects and especially to colours is the earliest clinical sign, but in Africans, particularly in those in the second stage of trypanosomiasis, detailed perimetry and campimetry tests are unreliable. Potter (1943) and Ridley (1945) found that there is relative sparing of the temporal as compared with the upper, nasal and lower quadrants.

After an interval of about two weeks, pallor, unaccompanied by swelling or by vascular abnormalities, appears in the optic discs and ushers in the third stage: the patients may become completely blind, having inactive three-quarter diluted pupils. Fourthly, there comes the stage of recovery which may continue for from three to six months. There may be a complete return of central acuity accompanied by some degree of improvement in the peripheral fields. The pallor of the discs remains unchanged or may even progress, the retinal vessels, especially the arteries, become somewhat constricted and white perivascular cuffing appears around the larger vessels near the optic discs. Acuity of even 6/5 is, however, compatible with almost complete pallor of the discs, but in such cases gross limitation of the fields will be found. In some cases recovery does not occur, the patient becoming permanently incapable of perceiving light ; the pupils are inactive and three-quarters dilated and the fundi show complete optic atrophy with constricted vessels and perivascular cuffing. Patients suffering from optic changes due to quinquevalent arsenicals may be divided into three groups : those with purely subjective and evanescent symptoms, those who end with a moderate degree of visual impairment and optic atrophy, and those in whom blindness is total and permanent and optic atrophy complete. According to Woods and Moore (1924), atoxyl amblyopia, unlike that due to tryparsamide, seldom improves when the drug is discontinued.

Histologically, Leinfelder (1938) finds acute degeneration of the retinal ganglion cells and of the innermost portion of the inner nuclear layer of the retina. According to Maclean and Fairbairn (1932), relapse cases are particularly susceptible to the toxic action of tryparsamide. Guyomar'ch (1932) has not been deterred from using tryparsamide, apparently with success, in patients already suffering from bilateral optic atrophy.

In diagnosing optic changes as due to tryparsamide, care must be taken to exclude the possibility of optic neuritis from nutritional defects and of eye changes caused by onchocerciasis.

In preventing optic changes the ideal would be to subject all patients to a thorough ophthalmological examination before treatment and after every injection. It is frequently stated that doses of tryparsamide up to 12 gm. are safe. This, however, is not the case. Woods and Moore (1924) found that 80 per cent. of toxic reactions arise during the first five injections and 94 per cent. during the first ten. In the Gold Coast it has been noted that even the first injections may be dangerous. Avitaminosis may be a predisposing factor, since patients in the later stages of sleeping sickness are very liable to suffer from malnutrition. It is usually thin and emaciated subjects who develop the most rapid ocular involvement. Barlovatz (1933b) believed that tryparsamide stored under tropical conditions for three years should not be used : it is probable that this period is too long and that greater safety is attained by using tryparsamide which has not been stored in the tropics for more than one year. The solution for injection must, of course, be freshly made up, and only glass-distilled water should be used.

In the diagnosis of optic atrophy in illiterates Landolt's rings are preferable to perimetry for testing acuity of vision, as they make less demand on the intelligence. Ideally, visual acuity should be tested before each injection (Scott, 1944).

Injections of sodium thiosulphate (ametox, thiostab) have been enthusiastically recommended by Raingeard (1934), but others have failed lamentably with this method of treatment. Forced drainage of the cerebrospinal fluid has been tried by Sloan and Woods (1936). Tryparsamide treatment is naturally discontinued, and it is probably unwise even after an interval to administer quinquevalent arsenic, although it may be necessary to choose between blindness and death from trypanosomiasis. If, however, disorders of the eye, including optic atrophy, are present before treatment begins and cannot therefore be due to arsenic, there is every reason for proceeding cautiously with injections. BAL has proved of very considerable value in treating other manifestations of arsenical toxicity, and there is every probability that its use will prevent or retard the development of optic atrophy. For adults, doses of 0·12 to 0·25 gm. of a 10 per cent. solution in oil may be given intramuscularly at four-hourly intervals : not less than three and probably not more than twelve injections are necessary. Ercoli and Wilson (1948), however, point out that BAL interferes much more with chemotherapeutic effect than with toxicity. The amount of BAL necessary to reduce the toxicity of a given dose of oxophenarsine (mapharsen) is 1·0 to 2·7 times as much again, whereas doses of BAL as low as one-eighth to one-half of the weight of oxophenarsine interfere with chemotherapeutic action.

Mora (1934), Assoreira (1934), and Ramos (1933) believed that tryparsamide, if injected directly into the carotid artery, was less likely to cause optic atrophy. Their results have not been confirmed.

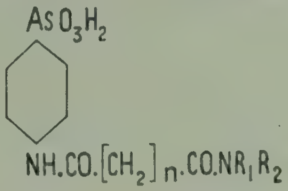
(2) **Sodium-4-acetylamino-2-hydroxylphenylarsonate.** Fourneau 270, or orsanine, was originally studied by Fourneau, Navarro-Martin, Tréfouel and Tréfouel (1923), who found it to have a curative action in experimental *Trypanosoma brucei* infections in mice, its chemotherapeutic index being 1 : 20. It was used clinically by Ledentu and Daude (1926), Ledentu and Vaucel (1927) and more recently by Sicé (1930, 1931 and 1933). It is generally believed by French workers that orsanine is equal to or even superior to tryparsamide in the treatment of patients in the first stage, 98 per cent. of early cases of Gambian sleeping sickness being cured. Though it acts more slowly than tryparsamide, it will also cure patients in the early stages of involvement of the central nervous system. In later cases of nervous involvement it will cure 78 per cent. of cases (Sicé, 1933). Its dosage, toxicity, and action on the optic nerve are closely similar to those of tryparsamide. According to Hawking *et al.* (1937), the power of orsanine to render the cerebrospinal fluid trypanocidal, after intravenous injection is as great as, if not greater than, that of tryparsamide. The greater activity of orsanine is due not to its greater penetration into the cerebrospinal fluid but to the larger proportion which is reduced within a given time to the active form.

(3) **Sodium-*p*-aminophenylarsonate.** Atoxyl (soamin sodium, arsanilate) was the first of the quinquevalent arsenicals to be used extensively. The introduction of drugs such as suramin, tryparsamide and orsanine has largely replaced atoxyl, but during the years 1939–45 it was still being used in French Gaboon. Vamos (1936) has given the results of treatment in early cases. Of 2,029 patients treated in the bush in the first stage, 1,172 were regarded as cured after observation periods of five to ten years. In thirty-four patients changes appeared in the cerebrospinal fluid but they were cured by tryparsamide : in 484 patients no nervous involvement had occurred in from one to four years. The average dose of atoxyl was 22 gm. in twenty-eight injections.

The widespread use of atoxyl in mass treatment seems to have been a factor in the production of naturally resistant arsenic-resistant strains of trypanosomes.

(4) **Sodium succinanilomethylamide-*p*-arsonate (neocryl).** To combine the desirable features of acetyl-*p*-arsanilic acid and tryparsamide an homologous series of compounds of the general formula $(HO)_2AsO . C_6H_4 . NH . CO(CH_2)_n . CO . NR_1R_2$ was prepared by Morgan and Walton (1931, 1937).

The chemotherapeutic indices of the compounds obtained by Morgan and Walton (1937) with the general formula of



is shown in the table.

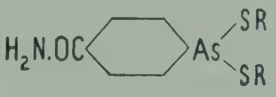
CHEMOTHERAPEUTIC INDICES

Compound.	Amide.	Methyl-amide.	Dimethyl-amide.	Ethylamide.	Prolyl-amide.
Oxalyl $n = 0$.	1	0	3	1	0
Malonyl $n = 1$.	1	1	1	6	4
Succinyl $n = 2$.	2	14	2	1	2
Glutaryl $n = 3$.	1	2	13	8	4
Adipyl $n = 4$.	4	4	7	4	5
Pimelyl $n = 5$.	2	3	2	2	2
Suberyl $n = 6$.	4	2	7	4	—
Azelayl $n = 7$.	1	1	—	0	—
Sebacyl $n = 8$.	1	1	0	—	—

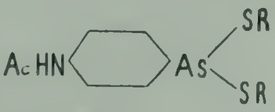
Among these compounds sodium succinanilomethylamide-*p*-arsonate ($n = 2$; $R_1 = H$; $R_2 = CH_3$), "neocryl," was selected for further study by Yorke and his colleagues (1936). The maximum tolerated dose of neocryl for a 20-gm. mouse is 40 to 50 mgm., the minimal effective dose for mice infected with *T. rhodesiense* 6 mgm. per 20 gm. of body weight and the minimum curative dose 12·5 mgm. as compared with 20 mgm. of tryparsamide per 20 gm. of body weight. In rabbits a dose of 1 gm. per kgm. of body weight killed four out of seven rabbits. In man, neocryl is well tolerated in weekly doses of from 2 to 4 gm. Lester (1935) tried it in eight cases of sleeping sickness in Nigeria and found that like tryparsamide it produced rapid clinical improvement, but its effect on the cerebrospinal fluid was erratic in the few patients in whom it was tried. Yorke *et al.* (1936) treated twelve patients with infections due to *T. gambiense* with promising results. Acres (1937, 1940), in the Belgian Congo, found that in first-stage cases there was definite clinical improvement as shown by lymph-node and lumbar puncture, the results comparing favourably with those produced by tryparsamide. In the second-stage, despite considerable clinical improvement, the results were unsatisfactory and within six months patients had relapsed. Only one case of optic atrophy was noted. This lack of toxicity to the optic nerve was stressed also by Ross (1940) in the treatment of 570 cases of neurosyphilis where the drug seemed to be as effective as tryparsamide. In the Gambia, Murgatroyd (1937) treated 122 patients of whom forty-four had normal and seventy-eight pathological cerebrospinal fluids. As a routine, ten doses of 45 mgm. per kgm. of body weight were given at weekly intervals, though in a few cases doses up to 60 mgm. per kgm. of body weight were administered. The drug was injected intravenously in a 25 per cent. solution in fresh glass-distilled water. Of forty-four first-stage patients one relapsed and two exhibited visual impairment but recovered. Of forty-six second-stage patients completing a course of ten or more injections, forty-five showed clinical improvement. Six patients suffered from toxic effects, three experienced visual disturbances and others had transient dizziness, vomiting and abdominal pain. In addition to the four patients who responded imperfectly to treatment four others relapsed in from seven to fifteen weeks after completing the treatment. The results are thus not as good as those with tryparsamide. This may possibly be correlated with the finding by Hawking *et al.* (1937) that though approximately the same amounts of arsenic are found in the cerebrospinal fluid after equal doses of neocryl and tryparsamide, yet the trypanocidal titre of the fluid is appreciable after tryparsamide but negligible after neocryl. It is suggested that the active compound found in the cerebrospinal fluid after a dose of tryparsamide is derived from a reduction of the drug in nervous tissue, whereas in the case of neocryl, although the drug reaches the brain, it is not converted into a trypanocidally-active compound.

(5) **Diarsonic Acids.** It has long been recognised that *Trypanosome congolense* fails to react to the quinquevalent arsenicals which readily cure other trypanosome infections in small rodents. This property of *T. congolense* is not due to any natural arsenic fastness, for Fourneau *et al.* (1933) showed that numerous compounds containing two arsonic acid groups can cure experimental infections due to *T. congolense*. The chemotherapeutic indices of these compounds varied from 1 : 1 to 1 : 3, the most active being derivatives of phenylenediarsonic acid of the type $NH_2C_6H_3{=}(AsO_3H_2)_2$, derivatives of diphenyldiarsonic acid, derivatives of *p*-aminophenylarsonic acid such as the substituted carbamide $CO(NHC_6H_4AsO_3H_2)_2$, and azo-compounds of the type $R{-}N{=}N{-}C_6H_4{-}C_6H_4{-}N{=}N{-}R$.

(6) **Arylthioarsinites.** In the thioarsinites tervalent arsenic is linked through sulphur to one, two or three organic radicals. A number of dithioarsinites capable of forming water-soluble sodium salts were prepared by Cohen, King and Strangeways (1931, 1932) by direct condensation of arylarsenious oxides with two molecular proportions of a thiol compound containing a carboxyl group. Starting with benzamide-*p*-arsenoxide and acetanilide-*p*-arsenoxide, two series of compounds (I) and (II) were prepared by employing in the condensations α-thiolacetic acid, β-thiolpropionic acid and its α-amino derivative, the naturally occurring amino-acid cysteine, *o*-thiobenzoic acid, *m*-thiolbenzoic acid, α-thiolacetamide and glutathione.



(I)



(II)

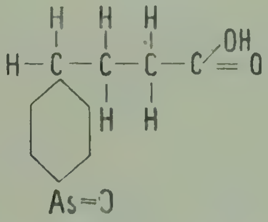
On testing the trypanocidal activity of a number of these compounds against *T. equiperdum* infections in mice it was found that as the molecular weight of the thiol constituent was increased, so the maximum tolerated dose, with one or two exceptions, also increased.

Of the arylthioarsinites prepared from cysteine and glutathione the most actively trypanocidal were shown by Strangeways (1935) to be di-(β-amino-β-carboxyethyl)-benzamide-*p*-thioarsinite, or K.324, and diglutathionyl-4-acetamido-2-hydroxy-phenylthioarsinite, or K.352. These compounds were effective in curing *T. rhodesiense* and *T. gambiense* infections in mice in doses which were only a fraction of the maximum tolerated. Three to six doses of 0·01 gm. of K.324 per kgm. of body weight, given at three-day intervals, produced permanent cures in rabbits infected with a strain of *T. rhodesiense.* In man, however, Murgatroyd (1937) found that even with doses of only 2·5 mgm. per kgm. of body weight vomiting and diarrhœa occasionally occurred, while with larger doses albuminuria was constant. In maximum tolerated doses no clinical improvement was seen, but one patient, after a single dose of 2·5 mgm. per kgm. of body weight of K.352 developed tachypnœa and hyperthermia and died.

(7) **Arsenical Derivatives of Anilinopyrimidines.** The trypanocidal action of the anilino-triazines (Friedheim, 1940a and b, 1944 ; Banks *et al.* 1944) has stimulated others to investigate analogous compounds. Because of the similarity between the triazine and pyrimidine nuclei, the arsonoanilino-pyrimidines and their related tervalent arsenic analogues were prepared by Banks and Controulis (1946) by the condensation of halo-pyrimidines with arsonanilines in acid solution : the trypanocidal activity of these compounds was inferior to that of arsenicals of the anilino-triazine series. Of the pyrimidines, 2-amino-4-(4′-arsonoanilino)-disodium pyrimidine, 2-amino-4-(4′-arsono-3′-hydroxyanilino)-disodium and 4-amino-2-(4′-arsonoanilino)-disodium pyrimidines were more active on *T. equiperdum* in rats than either atoxyl or tryparsamide but inferior to melarsen and melarsen oxide.

(8) **Phenylarsenoxides.** Re-examination of the chemotherapeutic properties of the arsenoxides has led to the use of *m*-amino-*p*-hydroxyphenylarsine oxide (oxophenarsine, mapharsen) in syphilis. Gruhzit (1935) found that in *T. equiperdum* infections in white rats it was as effective as neoarsphenamine, and though it was as toxic as the latter the sterilising dose was 1/12 and the chemotherapeutic index was double : its curative index was slightly higher (9 : 8·3) than that of neoarsphenamine. Swinyard *et al.* (1942) showed that small sub-curative doses were less effective the longer the time interval between the injections. King and Strangeways

(1942), in studying the relations between the chemical structure of various tervalent arsenicals and their action *in vitro* on normal and tryparsamide-resistant trypanosomes (*T. rhodesiense*), found that those phenylarsenoxides which contain carboxyl groups have the same lethal action on a normal and on a tryparsamide-resistant strain. The majority of substituted phenyl arsenoxides, as has been shown by Eagle *et al.* (1944), are not active trypanocidal agents. One compound, described as γ-(*p*-arsenophenyl) butyric acid (70 A, para-arséno or butarsen), however, was extremely active against trypanosomes (Eagle, 1945a and b) in experimental animals and man. Its synthesis is described by Doak *et al.* (1940) and its toxicity by Eagle *et al.* (1940). Butarsen, *p*-$OAsC_6H_4(CH_2)_3CO_2H$, is in reality an arsenoxide. It is a water-insoluble white compound



γ-(p-*arsenophenyl*)-*butyric acid*).

which dissolves in alkali to form a yellowish solution of the highly soluble sodium salt. The drug is packed as a stable dry powder in sterile rubber-stoppered vials, each containing 200 mgm. of the acid in the form of the sodium salt. This powder dissolves readily on the addition of 10 ml. of water to form the 2 per cent. solution generally employed for injection.

The usual dose in man is approximately 0·4 mgm. per kgm. of body weight per injection : for a man of 60 kgm. this works out at 24 mgm. given as 1·2 ml. of the 2 per cent. solution. The average dose of 0·4 mgm. per kgm. of body weight is 1/7 of the maximum tolerated dose in rabbits and 1/65 of the maximum tolerated dose in mice, but five times this dose level has been given to man for eight days without mishap. From seven to twenty-one injections have been given, their frequency varying from once daily to twice weekly. Eagle (1945a, 1946) records the treatment of 319 human cases of trypanosomiasis in West Africa, with observation periods after treatment up to eighteen months. In the majority of cases the drug was given intravenously, but in at least twelve patients the whole series of injections was given intramuscularly into the gluteal muscles: there was either no reaction or only transitory discomfort at the site of injection. The relative freedom from local reaction to this acid-substituted arsenoxide, in contrast to the marked inflammatory reaction after intramuscular injection of neoarsphenamine or oxophenarsine, is probably referable to the demonstrated lack of affinity between such acid-substituted arsenoxides and mammalian tissue cells (Eagle, 1945*b*; Hogan and Eagle, 1944). In acute infections in mice butarsen is about five times as effective as tryparsamide and

TOXICITY OF BUTARSEN AND THERAPEUTIC EFFICIENCY IN EXPERIMENTAL *T. equiperdum* INFECTIONS (Eagle, 1946).

A. TOXICITY

Number of injections.	Species.	Route of administration.	Maximal tolerated dose (LD<5) (mgm./kgm.).	LD 50 (mgm./kgm.)	LD 90 (mgm./kgm.).
Single injection	Mice	Intraperitoneal	26	33	50
	Rabbits	Intravenous	2·8	4·5	7·5
	Dogs	Intravenous	—	7·5±	—

B. THERAPEUTIC ACTIVITY

Species.	Treatment.	Total curative dose mgm./kgm.		" Chemotherapeutic index."	
		CD 50.	CD 90.	$\frac{\text{MTD}}{\text{CD 90}}$	$\frac{\text{LD 50}}{\text{CD 50}}$
Mice	Single injection, intraperitoneal . .	1·6	3·4	7·6	20·5
Rabbits	Four daily injections, intravenous . .	3·6	6·0	1·3	4·5

in chronic infections in rabbits about twice as active. One of the most remarkable effects of butarsen is the rapidity with which after a single injection of 0·3 to 0·6 mgm. per kgm. trypanosomes disappear not only from the peripheral blood-stream but from the cervical lymph nodes. In 88 per cent. of twenty-five persons examined thirty minutes after injection trypanosomes had disappeared from the nodes in 96 per cent. of twenty-three tested forty-five minutes after injection, and in 91 per cent. of eleven tested one hour after treatment. The remainder of the total of eighty-nine patients injected were negative within two hours of the injection. The blood also became free from trypanosomes in twenty-two patients in from thirty to sixty minutes after the first injection. This rapid action on trypanosomes *in vivo* is consistent with the fact that *in vitro* dilutions of 1 in 1,000,000 to 1 in 20,000,000 are found to immobilise the trypanosomes at room temperature in two to four hours (Eagle *et al.*, 1944 ; Eagle, 1945b) : these dilutions are of the same order of magnitude as those attained in the body fluids after an injection 0·4 mgn. per kgm. of body weight. Only one patient in the series described by Eagle (1946) was relatively resistant to the drug.

This compound appears to have relatively little toxicity for patients in the earlier stages of sleeping sickness. The characteristic nausea and vomiting so often seen after the injection of the arsphenamines or oxophenarsine were uncommon, occurring in less than 1 per cent. of cases. Extravasation at the site of intravenous injections caused slight local discomfort but less than that after oxophenarsine. One patient developed urticaria within thirty minutes of the first injection of 0·45 mgm. per kgm. of body weight, but the eruption disappeared rapidly and did not reappear on subsequent injection. A second patient developed jaundice after 4·7 mgm. per kgm. in twelve injections over thirty-six days. Two patients, a child and an adult male, developed encephalitic symptoms and spastic paralysis ; two deaths occurred, but were probably not due to the drug. No toxic reactions were seen even in seven patients who in error, instead of the usual dosage, received 2 mgm. per kgm. for five to ten days.

In early cases of infection with *T. gambiense* the final result is closely linked with the total dosage, as shown in the table on p. 380.

RESULTS IN EARLY TRYPANOSOMIASIS IN RELATION TO THE TREATMENT RECEIVED. (Eagle, 1946.)

Total dose of *p*-arsenophenylbutyric acid, mgm./kgm.		Observation periods; months							Totals.	Failures (%).	Apparent cures (%).
		<2	2–4	4–6	6–9	9–12	12–18	18 +			
< 3·5	Number of patients followed	21	11	7	3	1	—	—	43	26	74
	Number of failures . .	5	5	1	—	—	—	—	11	—	—
3·5–4·9	Number of patients followed	4	2	2	16	4	2	1	31	10	90
	Number of failures . .	—	—	—	3	—	—	—	3	—	—
5·0–6·4	Number of patients followed	15	4	9	8	1	—	—	37	8	92
	Number of failures . .	1	—	1	1	—	—	—	3	—	—
>6·5	Number of patients followed	11	18	39	5	15	—	—	88	5	95
	Number of failures . .	—	1	2	—	1	—	—	4	—	—

Early infections with *T. gambiense* are believed by Eagle (1946) to be curable within two weeks by twelve to fourteen daily injections of 0·5 mgm. per kgm., or within one week by six to seven injections of 1 mgm. per kgm. Where daily injections are not feasible the same number of injections may be given at any desired interval up to one week, apparently with equal therapeutic effect. In cases where the central nervous system is involved butarsen appears to be quite ineffective (Eagle, 1945b). These results were confirmed by Weinman (1946) and Weinman and Franz (1945). In three cases in the secondary stage, despite doses of from 380 to 400 mgm., the cell counts in the cerebrospinal fluid were higher at the end than at the beginning of treatment, and in one instance trypanosomes appeared in the cerebrospinal fluid during the course of treatment. On the other hand, Weinman found that ten patients in the early stages were cured so far as could be judged by an observation period of two and a half to eleven months. The total dosage was 360 to 400 mgm. for adults and in proportion to weight for children.

McLetchie (1948), in Nigeria, also found that butarsen is of value only in early cases. In advanced cases there seems little point in combining it with tryparsamide, and in fact this combination is less satisfactory than that of suramin and tryparsamide. Closely similar results were noted in French Equatorial Africa by Ceccaldi *et al.* (1948). With a few exceptions, trypanosomes disappeared from the blood and lymph nodes within fifteen minutes of injection, but of forty-two patients in the first stage ten relapsed in from twenty days to thirty months: four subsequently developed nervous symptoms and one patient died a week after receiving four injections in four consecutive days. In patients with involvement of the central nervous system the drug was of little or no value.

Apart from the rapidity with which it sterilises the blood and lymph nodes, the most interesting feature of butarsen is the fact that it can be used to treat cases which are resistant to arsenicals such as tryparsamide. In view, however, of its failure in cases where the central nervous system is involved, its action is obviously restricted to patients in the first stage when attacked by a naturally occurring arsenic-fast trypanosome (Eagle *et al.*, 1944 ; van Hoof 1947). When the cerebrospinal fluid cell count is less than 5 per c.mm. patients do well. In some instances in the second stage butarsen reduces the globulin content of the cerebrospinal fluid without necessarily reducing the albumin, the number of cells or the trypanosomes.

The effect of butarsen in a variety of infections due to trypanosomes is shown in the table.

THE CURATIVE DOSE OF *p*-ARSENOPHENYLBUTYRIC ACID IN A VARIETY OF TRYPANOSOMAL INFECTIONS (Eagle, 1946)

Species of trypanosome.	Animal host.	Curative dose (CD_{90}) of *p*-arsenophenylbutyric acid. mgm./kgm.
T. equiperdum	Mice	3·4 Arsenic-resistant strain equally susceptible 6·8 ±
	Rabbits	6·0
T. gambiense	Guineapigs	4·0 Arsenic-resistant strain equally susceptible
	Man	6 to 7.
T. rhodesiense	Mice	$>3·4$ $<6·8$
T. cruzi	Rats	After 2 mgm./kgm. temporary disappearance of trypanosomes from blood.
	Mice	No effect with 6·8 mgm./kgm.
T. congolense	Mice	No effect with 30 mgm./kgm.
T. evansi	Mules and horses.	Four of nine animals negative two weeks after six injections at 1·25 mgm./kgm. each, given every other day. Less intensive treatment ineffective.

Other observers (Mace *et al.* 1949) have found that against *T. evansi* in rats and dogs, doses of from 3·5 to 5·0 mgm. per kgm. produced only a 50 per cent. cure rate. In higher doses butarsen was toxic. Butarsen would appear to be no exception to the general rule in surra that early infections are much more easily controlled than late ones (Yutuc 1934).

Butarsen is not without its dangers ; an early sign of toxicity is an increase in the knee jerks : when this reflex is increased the drug should be stopped. In one Gold Coast case spastic paraplegia developed three months after the cessation of the sixth and last dose and after the appearance of this sign. The patient appeared to have disseminated lesions in the cortex and cerebellum. Butarsen should not be given intrathecally : a girl of eight years of age was given one intrathecal injection of 5 ml. of a 0·01 per cent. solution of butarsen ; five days afterwards the child was unconscious, incontinent, and suffering from unceasing convulsions. Seventeen days later the child could walk and eventually there was complete recovery except for paralysis of the right arm. A similar result occurred in another patient given half this dose.

In Nigeria, in human sleeping sickness, the effects of combining butarsen with small doses of tryparsamide have been examined : in twelve of fourteen patients in whom the total protein of the cerebrospinal fluid was under 30 mgm. per 100 ml. the results were good : when the total protein exceeded that figure the results were poor and were worse than with tryparsamide, especially in regard to the incidence of optic neuritis.

(9) **Azo-arsenobenzenes.** The action of the arsphenamines in trypanosomiasis has been limited by the fact that they are unable to traverse the blood-brain barrier. To attempt to overcome this difficulty Friedheim (1941a) combined arsphenamine with sulphonic acids. One of these preparations, 4197, is the sodium salt of 4 : 4′-dihydroxy-arsenobenzene-3 : 3′-bis [(azo-2) naphthol-(1)-disulphonic acid-4, 8]. It contains 13·8 per cent. of arsenic and is very soluble in water and insoluble in lipids. It can be prepared in a crystalline state and does not contain the *ortho*-amino-hydroxy and the amino-methylenesulphoxylate groups which are in large measure responsible for the instability of neoarsphenamine. It is stable indefinitely in the air at 18° C.

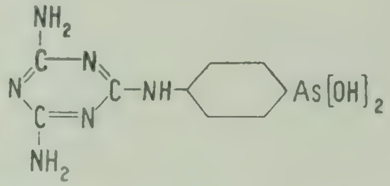
to 20° C., and under similar conditions solutions are stable for at least six months : at 37° C., however, solutions undergo changes and the toxicity increases. This compound is said, unlike the arsphenamines, to be able to traverse animal membranes : half an hour after the intravenous injection of 0·1 gm. per kgm. into a rabbit it can be found in the aqueous humour and six hours later in the cerebrospinal fluid. It is eliminated by the kidneys and colours the urine reddish violet. The maximum tolerated dose for the mouse intraperitoneally is 0·16 to 0·20 gm. per kgm. of body weight, and mice infected with *T. equiperdum* or *Borrelia recurrentis* are cured by doses of 0·008 to 0·102 gm. per kgm. of body weight. In rabbits the maximum dose tolerated intravenously is 0·125 gm. per kgm. : rabbits infected with *T. equiperdum* are cured by a single dose of 0·02 gm. per kgm. Dogs tolerate an intravenous dose of 0·08 gm. per kgm.

In 1939 Friedheim visited West Africa and tested the drug in cases of human sleeping sickness. The preparation used was a 5 per cent. aqueous solution : the usual dose was 0·5 gm., which was well tolerated not only by adults but also by children of 20 to 25 kgm. The dose was repeated eight or twelve times at intervals of two to four days. Doses of 0·75 gm. were tolerated by adults who were in good physical condition, and in one instance 0·9 gm. did not appear too great for an adult weighing 68 kgm. In patients in poor general condition, and in those with encephalitic lesions, the initial dose must be small (0·1 gm.), as otherwise there may be violent reactions of the Herxheimer type. In thirty-eight of forty-one cases trypanosomes disappeared from the blood and lymph nodes after the first, and in the remaining three cases after the second injection. The cell count fell as a result of the injections, but in only one case did it return to normal. Neither renal nor ocular lesions were noted, but though the immediate results were satisfactory the patients do not appear to have been followed up for long enough periods to determine whether they were really cured.

Muraz (1943a) found this compound and also another preparation prepared by Friedheim, azoarsinic acid 4196, less effective than melarsen.

(10) **Melarsen and Melarsen Oxide.** Friedheim (1940a and b,

1941a and b) prepared a series of compounds by condensing derivatives of triazine with phenyl arsinic acid. Of these the most important is 2,4-diamino-6-(arsono-anilino) triazine which has been called triazine arsinic acid, or melarsen : the corresponding oxide is known as melarsen oxide.



2,4-*diamino*-6-(*arsono-anilino*) *triazine* (4289).

The compound is also referred to as *p*-(2,4,diamino-*s*-triazinyl-6)-aminophenyl arsine oxide, and by French workers as 3177 R.P. In the form of its disodium salt it is a white crystalline powder containing 20·2 per cent. of arsenic, and soluble in water to make a 33 per cent. solution. When dissolved in propylene glycol the preparation of fractional doses is not always easy. In the dry form it is very stable and keeps for four months at a temperature of 65° C. : the solution is stable for three months at 45° C. It is unstable when autoclaved, and sterile solutions are therefore prepared by filtration. In mice intravenous injections of 0·75 gm. and 1·0 gm. per kgm. of body weight killed 10 and 20 per cent. of the animals, but given by mouth mice could withstand 10 gm. per kgm. of body weight. The minimum curative dose by mouth was 0·5 mgm. per kgm., the therapeutic index thus being 20. Rabbits tolerated 0·75 gm. per kgm. intravenously and dogs about 0·2 gm. per kgm. of body weight.

Triazine arsinic acid compared with tryparsamide as follows when given intraperitoneally to mice infected with *T. equiperdum* :

	Min. curative dose. gm./kgm.	Max. tolerated dose. gm./kgm.	Therapeutic index.
Triazine arsinic acid .	0·4	2·25	1 : 5·6
Tryparsamide . .	0·03–0·05	1·5	1 : 30·0

Experimental intoxication in mice does not produce the phenomenon of waltzing as is the case with tryparsamide. In West Africa (Friedheim, 1941b) it was found that the drug could be given either intravenously or subcutaneously. For patients in the first stage the dose at first recommended was 0·3 to 0·6 mgm. per kgm., with a maximum of 3 gm., every third day. In Senegal, where the population was well nourished, the interval between injections was two days. If the interval is decreased or the total dose increased there may well be gastro-intestinal troubles, vomiting, abdominal colic, and diarrhœa: occasionally dermatitis was seen. Ten injections over thirty days was the usual course. In the second-stage cases it is advisable to give a small initial dose, as the first injection is liable to be followed by a Herxheimer reaction. A week later, when the reaction is over, the normal dose can be tolerated. Thus the first dose was 0·1 mgm. per kgm., the second subcutaneous injection 0·2 mgm. per kgm., the third 0·3 mgm. per kgm., followed by 0·3 or 0·4 mgm. Of seventy-six patients treated parenterally all became negative after the first injection. In thirty-two instances peripheral sterilisation was observed twenty-four hours after the first inoculation. Twenty-nine cases were re-examined two and a half to nine months later and were clinically cured. In second-stage patients there was a considerable reduction in the number of cells in the cerebrospinal fluid but the quantity of albumin diminished more slowly. The clinical signs, however, improved rapidly. No evidence of optic atrophy was noted.

Later observations showed that rather larger doses could safely be given, and in man oral doses of 0·9 mgm. per kgm. every day for a week were well tolerated. Thirteen patients, nine in the first and four in the second stage, were therefore given 0·7 to 1·0 mgm. per kgm. of body weight daily for from two to three weeks. In ten patients the whole course of treatment was given, but in three instances symptoms of intolerance developed at the tenth, twelfth and thirteenth injections. Gastro-intestinal disturbances were present but no renal or visual disturbance. The effects of oral administration were slower than after injection, but in all cases the blood and lymph nodes were free from trypanosomes within three days. No relapses were seen. In the second-stage cases the number of cells and the albumin in the cerebrospinal fluid were considerably reduced. Muraz (1943a and b) also reported favourably on this preparation. Further investigations have been made in Liberia on melarsen oxide by Weinman and Franz (1945) and Weinman (1946), who gave seven daily doses. The oxide was given intravenously to nine patients at a dose of 0·10 mgm. per kgm. for seven consecutive days, the highest total being 46·2 mgm. in seven days. Three cases were in the second stage: one showed a relapse thirty-five days after treatment, one was not influenced and one showed a marked drop in the cell count. Of the patients in the first stage all showed sterile bloods and lymph nodes but only three could be followed for from thirty to sixty days after treatment: they, however, were all negative. Orally, melarsen oxide was given in gelatine capsules at a dose rate of 3·00 mgm. per kgm. daily for five to eight days. Four non-neurological cases were apparently cured seven to twelve months later. Of eight neurological cases all showed considerable improvement in the cell count in the cerebrospinal fluid, but two are known to have relapsed. Trinquier and Pellissier (1948a) found that two doses of 30 mgm. a day caused profuse diarrhœa. Fourteen daily doses of from 10 to 30 mgm., irrespective of body weight, were well tolerated and gave good results in early but not in later cases. van Hoof (1947) gave fifteen doses of 0·5 mgm. per kgm. every second day.

McLetchie (1948), in Nigeria, used both melarsen (2224 RP) and melarsen oxide. Melarsen oxide, although strongly trypanocidal, may be toxic in advanced cases, and in some early cases it seems to fail completely. In patients with nervous involvement the cerebrospinal fluid cell count is rapidly reduced, but after six months the relapse rate is as high as 1 in 3. Thrombophlebitis may occur, and among twenty-six cases with an average cell count of 58 per c.mm. of cerebrospinal fluid there were two cases of encephalopathy. When melarsen was given in doses of 1 gm. every five days for twelve injections, the average cell count in the cerebrospinal fluid being fifteen and the average total protein 21 mgm. per 100 ml., all patients were negative five months later. Although some observers, such as van Hoof (1947) in the Belgian Congo, believe that melarsen is too toxic, in Nigeria it has been found to give good immediate results. Trinquier and Pellissier (1948b) also had good results in early cases. They gave an oral dose of 0·20 mgm. per kgm. of body weight daily for two weeks. The dose was usually increased by 0·1 mgm. per kgm. daily till on the fifth day 0·6 mgm. per kgm. was being given. The total dosage was 3 gm.

From these observations it appears very doubtful whether either melarsen or melarsen oxide is suitable for the routine treatment of sleeping sickness in the field. The chief value of melarsen oxide seems to be in its action on strains of trypanosomes that are resistant to tryparsamide (van Hoof, 1947; McLetchie, 1948). The reasons for this activity against tryparsamide-resistant trypanosomes are discussed on p. 489. Friedheim (1948), however, as a result of more recent observations, has found that it is possible to use doses of melarsen oxide as high as 1·5 mgm. per kgm. of body weight intravenously in the form of a 5 per cent. solution in propylene glycol. A course then consists of two series of seven intravenous daily injections, the two series being separated by a rest period of one month. Fifty-four cases were thus treated, nineteen of them having signs of nervous involvement. The blood and lymph nodes were promptly sterilised and trypanosomes disappeared from all nine cases one week after a single series of seven intravenous daily injections. In thirteen out of thirteen cases the cell counts fell to normal three weeks after the first series of seven injections and in nine of thirteen cases it was normal even within one week of the first series of injections. The fall in the total protein content was less striking as in none of thirteen cases was there any reduction one week after the first series of seven injections: however, three weeks after the first course it had fallen to normal in four out of thirteen cases and one week after the second series of injections in four of the remaining nine cases. Clinical improvement followed the drop in cell count more closely than improvement in the protein content. Five advanced cases were followed up for from four to ten months after the last injections. Subsequent to treatment, the cell counts and protein contents of all patients reverted to normal but the reduction both in cell count and in total protein content was very slow, taking from three to nine months to reach normal. Improvement thus continues for a very considerable period after the cessation of all treatment.

These results are of considerable interest since, if confirmed, they suggest that the same drug may be used for the treatment of both stages of the disease and that melarsen oxide may be of great value also in the treatment of second-stage cases showing a resistance to tryparsamide and similar arsenicals.

"Mel B." A further melaminyl arsenical has been studied by Friedheim (1949b): "Mel B" is said to be an alkyl mercapto derivative of melaminylphenylarsenoxide. Its toxicity is considerably less than that of melarsen oxide, so that in the treatment of human patients the individual dose can be doubled and the period of treatment shortened. A single intravenous injection consists of from 2 to 4 mgm. per kgm. of body weight and the total number of injections recommended is from three to fourteen: mel B is used in the form of a 5 per cent. solution in propylene glycol.

Trypanosomes were removed within twenty-four hours from the cervical lymph nodes of twelve cases by an intravenous dose of 1·5 to 4 mgm. per kgm. of body weight. Similar findings were recorded for fifty cases with trypanosomes in lymph nodes before treatment; in eight cases where trypanosomes were originally present in the cerebrospinal fluid they could not be found seven days after four intravenous injections of 3·6 mgm. per kgm. of body weight each, given at the rate of one injection for four consecutive days.

In all, fifty cases of second-stage sleeping sickness have been treated. In twenty cases, forming group 1, the cell and albumen-content of the C.S.F. returned to normal in an average control period of 108 days: the number of injections varied from three to fourteen; individual doses varied from 1·9 to 4 mgm. per kgm.: in group 2, twenty-three cases, the cell count but not the albumen-content had returned to normal after a control period averaging seventy-eight days. In group 3, containing seven cases, both cell counts and albumen-content were still above normal after a control time averaging seventy days. The cell count, however, was significantly reduced but the albumen-content was stationary or only slightly reduced. No toxic effects were recorded. It is considered that after the first three or four injections there should be a rest period of a week to avoid possible Herxheimer reactions.

(11) **Aromatic Arsonic Acids containing Amide Groups.** Gough and King (1930) found that benzamide-*p*-arsonic acid was trypanocidal in mice but produced very severe nervous symptoms. A series of arsonic or arsinous acids containing carboxyl or sulpho groups was inactive but, when converted into primary amides, carboxyamides or sulphonamides, trypanocidal activity appeared in all cases. This is explained by the fact that the acids when containing sulpho or carboxyl groups are rapidly excreted, whether reduced to the arsenoxides or not, since they contain solubilising groups. Those compounds containing amide groups will be excreted rapidly so long as they contain arsonic acids, but when once reduced to the arsinous acid stage excretion will be slow, since the solubilising group, the arsonic acid group, has disappeared.

(12) **" Arseno-Detoxin " Compounds.** What are claimed to be compounds consisting of the condensation products of hydroxyamino- and hydroxyamino-benzylarsen-oxides and their substitution products with high molecular keratin hydrolysates and thus containing sulphur have been studied by Collier and Krause (1935) and Collier and Verhoog (1937). One compound, " As XIII," was found to have a chemotherapeutic index of 1 : 66·6 to 1 : 80 when injected subcutaneously into mice infected with nagana. When given by mouth, its chemotherapeutic index was only 1 : 10.

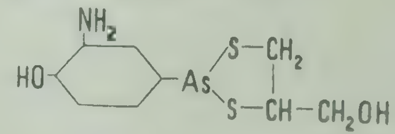
(13) **Arsenical Derivatives of Pyridine.** Twenty-one arsenopyridines and two arsenostibio compounds were studied by Christison (1934) for their action on infections due to *T. brucei* and *T. lewisi.* One compound, BR 23, 2-pyridone-3-amino-5-arsonic acid, had a weak action on *T. lewisi.* The arsenostibio-compound Sdt 386 B, so active in *Bartonella* infections, has only a very slight action on *T. lewisi.* Lester (1935), in human cases due to *T. gambiense*, found that it was much less active than suramin.

(14) **Arsenic-containing Dyestuffs.** Two arsenic-containing dyestuffs, arsenic yellow and arsenic brown, have been reported by Fischl and Singer (1935) to have some action on mice infected with *T. brucei* as well as on infections due to *Borrelia recurrentis.*

(15) **Silver Arseniate.** A silver arseniate has been used in French Gabon by Pierron (1947), who claims excellent results with daily intravenous injections for thirty days. The doses given are only one hundredth those of the usual arsenicals: the toxic reactions are said to be slight and no instance of blindness has been seen. In addition, it is possible to use this compound in patients who carry trypanosomes resistant to arsenic. In the first stage the blood becomes sterile in from five to six days and enlarged cervical lymph nodes disappear in about thirty days, while clinical symptoms show improvement within a week: forty-eight cases in the first stage and nineteen in the second stage are all said to have been cured: the period of surveillance was probably inadequate. Arsenic trichloride was found by Dubois (1942) to have a curative action on *T. congolense* infections in mice: it is, however, toxic.

BAL and Organic Arsenical Derivatives

While 2,3-dimercaptopropanol (British Anti-Lewisite or BAL) protects man and experimental animals against the toxic action of arsenicals such as oxophenarsine hydrochloride (mapharsen), it also reduces very considerably the trypanocidal effect (Peters *et al.* 1945; Waters and Stock, 1945, and Sulzberger and Baer, 1947). Pfeiffer *et al.* (1947) have shown that the reversal of the chemotherapeutic effect of oxophenarsine can be demonstrated for at least three hours after apparent cure. If rats infected with *T. equiperdum* are given 5 mgm. per kgm. of oxophenarsine intravenously apparent cure takes place: if 75 mgm. per kgm. of body weight is given three hours later a relapse occurs and trypanosomes appear once more in the peripheral blood stream. The effect of condensing BAL with oxophenarsine hydrochloride was studied by Riker (1946), Peters and Stocken (1947), and Friedheim and Vogel (1947), who produced 2-amino-4-[methylol-(ethylene-dimercaptoarsino)]-phenol (I).



(I)

This compound, known as BAL-OXO, crystallises from methanol and forms a crystalline hydrochloride, quite stable and soluble in water, ethanol and propylene glycol, but insoluble in anhydrous acetone. Propylene glycol solutions of the hydrochloride can be sterilised by heating for one hour at 100° C. The hydrochloride cures experimental *T. equiperdum* infection of the mouse with a single intraperitoneal dose of 0·02 gm. per kgm., whereas the maximum tolerated dose, intraperitoneally, is 0·12 gm. per kgm., corresponding to a therapeutic index of 1 : 6.

Similar results have been obtained with analogous BAL derivatives of other aromatic arsenicals as well as with aromatic antimony compounds, including arsanilic acid, tryparsamide, carbarsone, stibanilic and acetyl stibanilic acid. Hence, generally speaking, inclusion of a phenyl-substituted arsenic or antimony residue in a five-membered, sulphur-containing ring as in (I) is not incompatible with a significant chemotherapeutic activity.

The mechanism of the therapeutic effects of BAL derivatives is at present undetermined. There are at least two possibilities: (1) the molecule may act as a whole ; this necessarily implies a mechanism not foreseen by the Ehrlich-Voegtlin SH-arseno-receptor theory ; (2) BAL compounds may be dissociated in the organism and act essentially like the parent compounds, simply altering the distribution of active arsenic between trypanosomes and host. The latter mechanism is probably more in keeping with what is known of the mechanism of arsenicals. If the dithioarsenite were to act as a whole it could not be through the —SH arseno-receptor groups.

There is still an unexplained discrepancy as to the relative toxicity of BAL-OXO and oxophenarsine hydrochloride. Peters and Stocken (1947), using a propylene glycol solution of the hydrochloride of BAL-OXO found that in rats it was approximately four times as toxic as the parent arsenoxide, the LD 50 being from 1 to 2 mgm. As per kilo of body weight. Riker (1946) found that in cats BAL-OXO was at least four times less toxic than the parent arsenical. Friedheim and Vogel (1947) gave toxicity figures for BAL-OXO in the mouse and rabbit which were considerably less than those provided by Eagle *et al.* (1944) for oxophenarsine hydrochloride, and Sawyers *et al.* (1949) found that the acute toxicity of the thioarsenite, BAL-OXO, is approximately one-quarter that of the oxophenarsine hydrochloride from which it was prepared. Rats survived 32 mgm. As per kgm. of body weight and died at 64 mgm. As per kgm. of body weight.

The chemotherapeutic index of BAL-OXO is, however, only about one-third that of oxophenarsine hydrochloride since its activity against trypanosomes is reduced less than its toxicity for the host.

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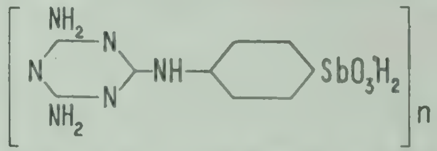
Antimony Preparations

Antimony was first used in the treatment of trypanosomiasis by Plimmer and Thomson (1908), who found that the blood of laboratory animals infected with *T. brucei* or *T. evansi* was sterilised by injections of potassium or sodium antimonyl tartrate. Mesnil and Brimont (1908), and Broden and Rodhain (1908) obtained similar results. Kérandel (1910), who acquired an infection with *T. gambiense*, was treated successfully with intravenous injections of tartar emetic. Until recently antimony was almost the only substance widely used in the treatment of trypanosomiasis due to *T. congolense* in domestic animals (Hornby, 1919; Bevan, 1928; Schwetz, 1933). The chief drawback to the use of tartar emetic is that owing to its irritant action it must be given intravenously. Fulton and Yorke (1943) compared the action of tartar emetic on *T. congolense* in mice with that of other compounds: tartar emetic was low on the list but numerous attempts have been made to introduce other antimonial compounds which might be suitable for the treatment of human trypanosomiasis.

Infections due to *T. vivax* and *T. uniforme* were treated with success by a tervalent antimonial, antimosan, a complex derivative of tervalent antimony with pyrocatechin disulphonate of potassium, while the same drug has cured a sheep experimentally infected with the porcine trypanosome *T. simiæ* (Hornby, 1937); *T. congolense* infections in cattle were also cured. When given subcutaneously, however, the drug is very irritating, and when given intravenously less effective (Curson, 1926; Hornby, 1930; Du Toit, 1930; Parkin, 1930; Schwetz, 1933; Stewart, 1935. and Evans, 1936). The usual course is 10 ml. per 100 lb. body weight once a week for five weeks. Launoy and Prieur (1937) studied the trypanocidal action of four organic preparations of antimony, probably complex tartrates of stibonic acids and described as *p*-aminophenylstibonate of methyl glucamine, sodium diphenylcarbamide stibonotartrate, sodium *para*-acetylaminophenylstibonotartrate and sodium diphenylcarbamidodistibonopyrocatechol sulphonate. Methylglucamine aminophenylstibonate which contains 30·3 per cent. of antimony was the only one which was found to have a curative action against *T. brucei* and *T. annamense*. To cure *T. congolense* infections twice the amount of antimony required for the other trypanosomes was necessary. Stibophen has some action on *T. congolense* infections in cattle, but four to five injections at weekly intervals are necessary (T-W-Fiennes, 1947). In addition to the methylglucamine salt, glucamine aminophenylstibonate was found to be active by Launoy (1937), who investigated also the trypanocidal action of lithium antimony thiomalate (anthiomaline), a tervalent antimonial. In early infections of mice due to *T. annamense* this drug shows considerable activity, but in later infections only about half the mice are cured, and in guinea pigs the only action is a slight prolongation of the infection ; no cures are obtained.

A number of antimonial compounds of hydroxyquinoline sulphonic acid were prepared in Belgium and a few have been tested in sleeping sickness. Four of the compounds, Dn 9, Dn 12, Dn 17 and Dn 20, are described by Rodhain *et al.* (1933) as quinquevalent antimonials, the remainder, Dn 7, Dn 8, Dn 11, Dn 14 and Dn 18 as tervalent. All are trypanocidal but, apart from Dn 9 and Dn 18, are so irritating that they cannot be given subcutaneously to animals. van Hoof (1933a and b) treated twenty-six patients with Dn 12. Doses of 0·5 gm. were given intravenously twice weekly to a total dose of 5·0 to 10·0 gm. Many patients were unable to tolerate such large doses and individual doses were therefore reduced to 0·25 gm. Immediate shock and toxic symptoms as the result of accumulation of the drug were not uncommon, and dermatitis occurred in four of the twenty-six patients. In some patients trypanosomes disappeared from the blood and the cells in the cerebrospinal fluid were reduced in numbers ; in others, however, no curative effect was produced. Dn 18 is said to have a definite curative value in advanced cases and to be active in cases where the trypanosomes have become resistant to quinquevalent arsenicals. *T. congolense* infections in cattle are said to react rapidly, but the drugs are irritating when given subcutaneously and less effective intravenously.

During the war years, when the increase in the number of patients with arsenic-resistant trypanosomes increased rapidly in the Belgian Congo, tartar emetic was used in conjunction with an arsenical (Haveaux, 1945 ; Eeraerts, 1946). It is questionable whether such treatment is advisable, for van Hoof (1947) has pointed out that resistance to tartar emetic is now more prevalent where arsenic resistance is most common, that is to say where arsenicals have been much used. This result might have been expected, since Yorke *et al.* (1932) showed that though it was very difficult to enduce resistance to tartar emetic alone, yet if tartar emetic is combined with arsenicals, resistance to tartar emetic arises very readily. Another antimony compound has been prepared by Friedheim and Berman (1946) : it is a sodium salt derived from *p*-(2,4-diamino-1, 3, 5-triazinyl-6) aminophenylstibonic acid and is said to have a structural formula corresponding to



where *n* is greater than 3. It is freely soluble in water. Mice tolerate an intraperitoneal injection of 2·5 gm. per kgm. of body weight, and a single injection of 0·125 gm. per kgm. of body weight is said to eradicate *T. equiperdum* infection in mice even when the drug is given twenty-four hours after intraperitoneal injection of the trypanosomes. The drug is also of use prophylactically for, after a single intraperitoneal injection of 0·05 gm. per kgm. of body weight, 100 per cent. of mice were protected for fifty days, and even eighty-five days after injection 95 per cent. of animals were protected, probably because in the body free stibonic acid is deposited at the site of injection. It is believed by Mayer and Brousseau (1946) that the long periods of protection such as 330 days afforded to some mice is due to the fact that all trypanosomes are not immediately killed off but that some survive and produce an active immunity. Browning and Gulbransen (1936) thought that acetarsol had a similar action.

Goodwin (1944) tested the trypanocidal activity of a number of ter- and quinquevalent antimonials in mice by two methods, one depending on the removal of trypanosomes from the peripheral blood stream, the other on the survival times of infected mice given different doses of the drugs. Both methods give reproducible results and have the same variance, but there are differences with phenyl stibonic acid derivatives showing that the two methods measure different types of activity. It is thus not possible to give a single figure to express the ratio of activities of a pair of substances so different in their mode of action as tartar emetic and stibacetin. Speaking broadly, toxicity, irritancy on subcutaneous injection, and trypanocidal activity are related but are independent of antimony content. The more rapidly excreted substances are less toxic and less active than the others. Neostibosan and the quinquevalent analogue of tartar emetic had little or no trypanocidal action when given in a single dose but, as Bülbring and Burn (1938) found in the case of neostam, activity can be shown if these compounds are given repeatedly at short intervals. However, after repeated small doses relapses always occur (Fulton and Yorke, 1943). Chen, Geiling and MacHatton (1945a) also made use of survival times in investigating the action of antimonials on *T. equiperdum* in mice, obtaining various indices $\frac{\text{LD 50}}{\text{CD 50}}, \frac{\text{LD 10}}{\text{CD 90}}, \frac{\text{LD 5}}{\text{CD 95}}$. The results are shown in the table. Derivatives of phenyl stibonic

Chemotherapeutic Index of Antimonials in Infections due to *T. equiperdum* in Mice (Chen, Geiling and MacHatton, 1945a)

Compound.	$\frac{\text{LD 50.}}{\text{CD 50.}}$	$\frac{\text{LD 10.}}{\text{CD 90.}}$	$\frac{\text{LD 5.}}{\text{CD 95.}}$
Tartar emetic . . .	1·21	0·66	0·56
Sod. antimony thioglycollate	1·67	0·81	0·66
Anthiomaline . . .	1·44–1·49	0·73	0·58
Stibophen . . .	3·74	1·21	0·87
Stibamine . . .	2·90	1·46	1·35
Stibenyl . . .	3·57	1·53	1·43
Neostibosan . . .	1·74–2·08	0·93–1·03	0·77–0·90
Neostam	1·75	0·85	0·71

LD 50 = Dose killing 50 per cent. of animals.
CD 50 = Dose curing 50 per cent. of animals.

acid were the most active therapeutically. The therapeutic activity of antimony derivatives was increased if treatment was begun twenty-four hours after infection: the same thing has been noted with arsenicals (Kolmer, 1931). Chen *et al.* (1945b) found that cysteine reduced both the trypanocidal activity and toxicity of tartar emetic, stibophen, anthiomaline and sodium antimony thioglycollate but not of stibamine, stibenyl, and neostibosan. *In vitro* cysteine antagonises the inhibitory effect of both stibamine and tartar emetic on the glucose metabolism of trypanosomes (Chen and Geiling, 1948).

Another method for determining the antitrypanosome effects of antimonials has been worked out by Chen and Geiling (1945). Trypanosomes isolated from blood elements by centrifugation are suspended in a phosphate buffer. The rate of metabolism of glucose for one hour at 37° C. is determined before and after the addition of antimonials. Comparisons are based on the concentration required to give a 50 per cent. inhibition.

BAL may be used to decrease the toxicity of antimonial drugs, but trypanocidal action is also decreased.

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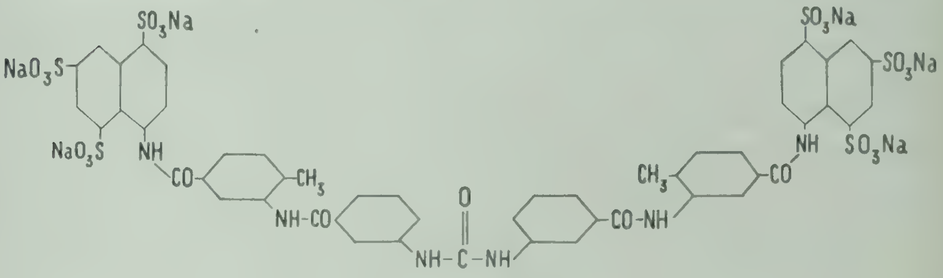
NON-METAL-CONTAINING COMPOUNDS

Suramin

Suramin, another drug which has suffered from a multiplicity of names, is known also as germanin, Bayer 205, moranyl, Fourneau 309, belganyl, naphuride, naganol, and antrypol.

Its chemical constitution is given by Schlossberger (1928) as :—



(*I*) *Suramin*

a structural formula identical with that arrived at by Fourneau (1924). As far as is at present known, the slightest deviation from this formula is attended with a diminution of trypanocidal activity. This explains why other ureas of the aminonaphthalene-sulphonic acid series have not found a place in the chemotherapy of trypanosomiasis.

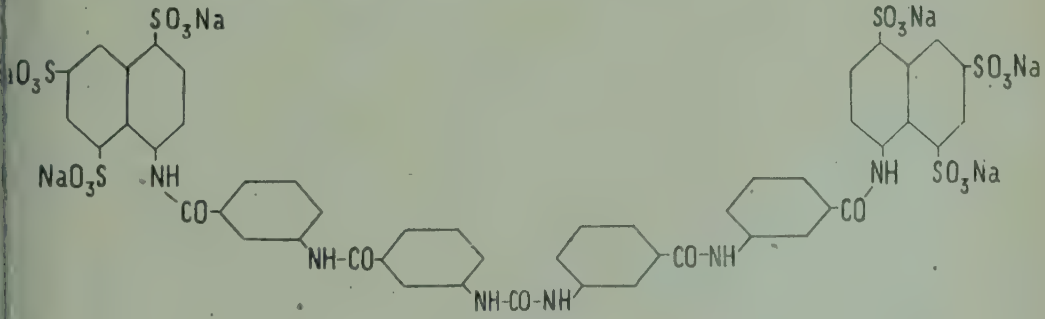
Adequate chemical methods of estimating suramin in body fluids were devised by Steppuhn and Utkin-Ljubowzow (1924), Lang (1931), Dangerfield *et al.* (1938), Boursnell *et al.* (1939) and Vierthaler and Boselli (1939). Dangerfield *et al.* (1938) showed that methyl-α-naphthylamine produces a magenta colour when coupled in acetic acid solution with the diazotised products of hydrochloric acid hydrolysis of germanin.

Suramin displays an extraordinarily marked persistence in the blood of man and animals. This property was first recognised, soon after suramin was introduced, from the long duration of its prophylactic action (Mayer and Zeiss, 1920; Levaditi and Klarenbeck, 1927); from measurements of the trypanocidal activity of serum and urine in treated animals (Mayer, 1922 ; Mayer and Menk, 1922) ; and from direct determination of its presence in the blood stream (Lang, 1931 ; Dangerfield *et al.*, 1938 ; Boursnell *et al.*, 1939 ; Vierthaler and Boselli, 1939 ; Hawking, 1940a and b). By these tests it was found that suramin may persist in the blood stream of man, dog, and rabbit for as long as three months. The form of the blood concentration curve is asymptotic (Lang, 1931) ; during the first few hours after intravenous administration the concentration falls rapidly but thereafter more and more slowly, until it may be regarded as almost constant over periods of a few days. Town and Wormall (1949) report that in rabbits the intravenous injection of 28 mgm. of suramin per kgm. of body weight, equivalent to 2·0 gm. for a man of 70 kgm. body weight, produced a plasma suramin level of above M/2,000 for four minutes, above M/3,000 for twenty-five minutes, above M/5,000 for eighty minutes, and above M/10,000 for seven hours.

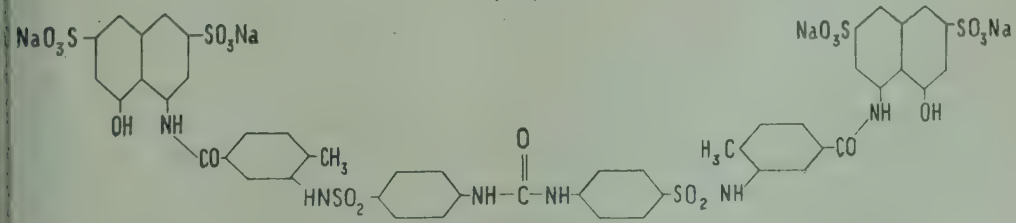
In view of its use in the prophylaxis of trypanosomiasis in the field the reasons for this persistence are of considerable interest. No depôt of suramin appears to be formed in any tissue and tissue concentrations are of the same order, or lower than, the plasma concentration (Zeiss and Utkin-Ljubowzow, 1930 ; Boursnell and Wormall, 1939). It is probable that the kidney excretes suramin only very slowly although it can be detected in the urine a few days after administration (Boursnell *et al.*, 1939). The slow excretion is generally believed to be due to the fact that suramin is bound to protein either in plasma or cells. Mandel and Steudel (1926) suggested that it was combined with basic proteins of the histone type as such combinations can occur *in vitro*. Collier (1926, 1927) showed that it could combine with globulins, Brunelli (1934) demonstrating that it combined with the euglobulin fraction of horse serum and to a less extent with the pseudoglobulin fraction. Boursnell and Wormall (1939) considered that proteins other than globulins might be involved, and this was in fact demonstrated by Dewey and Wormall (1946), who showed that combination occurs within a few minutes. Wilson and Wormall (1949) find that complexes containing large amounts of firmly bound suramin can be precipitated by the addition of dilute acid to solutions containing suramin and proteins. Smaller but nevertheless significant amounts of the drug are firmly attached to the serum proteins at *p*H 7·5. The capacity of suramin to combine firmly with proteins is dependent on the naphthylamine-trisulphonic acid groups of the molecule. The —SH groups of the protein molecule do not play any significant part in this combination. It is probable that suramin combines also with trypanosomes, since trypanosomes exposed to suramin and then washed are no longer infective for animals though they remain active *in vitro* for over twenty-four hours at 37° C. (Hawking, 1939).

Other factors, however, may be involved in the slow excretion of suramin, for many drugs bound to protein are rapidly eliminated, their slow filtration through the glomeruli being augmented by participation of the tubular epithelium in the renal excretory processes. Examples are sulphathiazole, *p*-aminobenzoic acid (Lundquist, 1945) and penicillin (Beyer *et al.*, 1944 ; Rantz *et al*, 1944 ; Beyer, 1947). Conversely, the more persistent sulphonamides are those that are extensively reabsorbed from the glomerular filtrate by the tubular epithelium (Fisher *et al.*, 1943 ; Earle, 1944) : usually they are bound to plasma protein. Tubular processes may also play a similar part in the persistence of suramin. In addition the strength of the bond uniting it to protein may be of importance (Gregerson and Rawson, 1943 ; Rawson, 1943). A further possibility is that suramin exists in solution as colloidal aggregates of sufficiently high nominal molecular weight to hinder passage through the glomerulus. Some support for this suggestion is provided by the known inability of suramin to pass through a collodion membrane (Boursnell *et al.*, 1939). Suramin cannot pass through the wall of the red blood cell although it readily combines with hæmoglobin as in laked blood. Thus under normal conditions none of the blood suramin after injection is combined with hæmoglobin (Wilson and Wormall, 1949).

The prophylactic action of suramin is associated with the maintenance of a blood concentration of about 1·3 mgm. per 100 ml. as determined by the Wormall method (Vierthaler and Boselli, 1939) : it is possible that part of this concentration is contributed by degradation products, and as suramin is a polyamide related in structure to the natural polypeptides it might be hydrolysed to the three constituent aromatic amino-acid residues. Spinks (1948), however, could not obtain any evidence that suramin is hydrolysed *in vivo*, while Dewey and Wormall (1946) found that a mixture of amino-acid residues prepared by acid hydrolysis of suramin was rapidly eliminated. By preparing eleven compounds related to suramin, Spinks (1948) has shown that in the rabbit marked persistence in the blood is a property of polyamides of high molecular weight which contain naphthylamine-sulphonic acids as end-groups: the presence of two naphthalene polysulphonic acid groups is essential for persistence; *m*-amino-*p*-toluic, *m*-aminobenzoic, and sulphanilic acid groups may be interchanged in the molecule without marked effect on the persistence; a phenolic group can be introduced and the shape and spacing of the molecule may be much modified without any marked effect on persistence. It seems probable that any symmetrical compound of high molecular weight with a polysulphonic acid as an end group will show strong persistence. Thus some azo dyes of high molecular weight derived from naphthalene sulphonic acids such as trypan blue or Evans blue show strong persistence, but the susceptibility of the azo linkage to enzymic reduction probably accounts for the fact that they are not retained in the body quite so long as suramin.



(*II*)



(*III*)

Persistence of suramin is not related to trypanocidal activity. Persistence and activity are due to different structural features of the molecule. for compound (II) is of low trypanocidal activity (Davey, 1943) and compound (III) has but a trace of activity (Browning, 1939) but persistence is equal to that of suramin. The inactivity of many compounds prepared by Fourneau *et al.* (1924) which would almost certainly be highly persistent also shows that activity and persistence are not related. As hydrolytic products are rather rapidly eliminated it may be assumed that *in vivo* suramin is not hydrolysed or is hydrolysed only very slowly. It may of course be oxidised to phenols, phenolic sulphates or glucuronides, but the long duration of prophylactic action and the known delicacy of the balance between structure and activity (Fourneau *et al.*, 1924) strongly suggest that suramin is relatively resistant to metabolic processes.

Toxicity of Suramin. In the evaluation of any chemotherapeutic remedy the question of toxicity is one of considerable importance.

Suramin is not without toxic action, more especially in very poorly nourished persons. Lourie (1942), among the almost famished Kissi tribe in Sierra Leone, found a high percentage of reactions, thirty of 148 patients having a considerable reaction, including fever, headache, generalised body pains, cough, bronchitis, papular rashes, localised œdema, especially of the face and legs but sometimes of the arms and scrotum, peeling of the skin, especially round the mouth, blepharitis, conjunctivitis, itching of the skin and jaundice. Harding and Hutchinson (1948), also working in Sierra Leone, had many fewer reactions. Reactions following suramin may be divided into (1) immediate, (2) delayed, the delay being either of a few hours or of some days.

Immediate Reactions. The most common is nausea. Among 3,572 persons injected by Harding and Hutchinson (1948) vomiting was seen in 0·45 per cent. Fain (1942) found that in twelve of 4,500 persons receiving suramin nausea was associated with vomiting, a condition of shock, and loss of consciousness. The symptoms of collapse may be preceded by a short period of motor excitement and congestion sometimes involving the whole body, at other times confined to the face. The period of congestion is followed by sweating and by circulatory collapse. Harding and Hutchinson (1948) noted mild syncope in 0·14 per cent. of cases. The whole incident suggests a nitroid crisis rather than an ordinary fainting attack. It appears to be much more common in adult females than in males.

The incidence of vomiting after suramin is much reduced by giving the injection very slowly. The adoption of the recumbent position is valueless and it is better while giving the injection to have the patient sitting up in a well-ventilated position and to put cold compresses on the forehead. Caffeine has some action in overcoming the tendency to collapse.

Colic is rare after suramin and is more common after pentamidine. It occurred in 0·8 per cent. of the series recorded by Harding and Hutchinson (1948). Some slight rise in temperature usually occurs within half an hour, and various skin eruptions of an urticarial nature have been seen within one hour of injection. Harding and Hutchinson (1948) noted them in 0·2 per cent. of cases. Sometimes the papules are large and flat, rounded but of unequal size, at other times the papules are small and acuminate.

Late Reactions. A few hours after injection papular eruptions may be seen, sometimes pruriginous and accompanied by paræsthesiæ over the whole body. Fever may appear within two or three hours after injection and may reach 40° C. Some patients show a slight febrile reaction, continuing for some days after injection. Intense photophobia and lachrymation, sometimes associated with palpebral œdema, have been noted twenty-four hours after injection. Other toxic symptoms are abdominal distension and constipation, and cutaneous hyperæsthesia, localised to the distal parts of the limb. This symptom is specially liable to occur in adults and is seen on the soles of the feet or palms of the hand. The pain, which comes on twenty-four to forty-eight hours after injection, may persist for a week or more and in the worst cases the skin may desquamate : when the feet are involved walking becomes difficult.

Delayed Late Reactions. Of forty-six Africans in the Belgian Congo given suramin by Fain (1942) twenty-four showed some degree of albuminuria but in only four was there a high degree of albuminuria associated with granular casts and red blood cells.

These signs were noted a week after inoculation and lasted for about five days. Anuria has occasionally developed. Albuminuria may sometimes persist for a considerable period. It must, however, be remembered that albumin in the urine of Africans with sleeping sickness is by no means rare. De Marqueissac (1933) noted albumin in the urine of twenty-five of thirty-three untreated patients. After a few injections of suramin the albumin had either disappeared or was reduced in amount.

Late toxic reactions appear to be especially common among those who have lived on a starvation diet.

Occasional deaths have been recorded, though the proportion is not greater than 1 in 1,000 (Lester, 1935, 1938, 1945 ; Fain, 1942). Solutions of suramin left for twenty-four hours or more at room temperature in the tropics appear to increase in toxicity. Cockburn (1947) records the distressing symptoms following the intravenous injection of such a toxic preparation.

Intramuscular injections may give rise to considerable pain and necrosis. Absolute sterility must be ensured, as at least one fatal case of gas gangrene is known to have occurred as a result of an intramuscular injection of suramin. Patients with albuminuria need not give up suramin injections unless other signs of kidney dysfunction appear ; this applies to infections due to *T. rhodesiense* as well as to *T. gambiense*. In rabbits infected with *T. annamense* where the trypanosome itself causes albuminuria, suramin completely cures the infection (Launoy, 1944). Ramos (1933) gave intracarotid injections and claimed good results.

Although suramin given in the first few weeks of illness due to either *T. gambiense* or *T. rhodesiense* may cure some patients, neither suramin, pentamide nor butarsen can be used alone unless a large staff is available to perform lumbar punctures on all patients and to eliminate all those with nervous involvement. The after-history of patients treated with suramin alone is not, in fact, impressive. Keevil (1934) followed up for eight years six patients who originally showed *T. rhodesiense* in the cerebrospinal fluid : three were alive and well and three had died after two and a quarter, five, and eight years : the cause of death was not sleeping sickness in one case and was unknown in the two others. Saunders (1942) reported the after-history of thirty-six patients with infection due to *T. gambiense* treated with suramin alone thirteen years or more previously. Only one, an early case, was cured, another, a late case, was still alive but had relapsed, and the other thirty-four had died. Nevertheless, in mass treatment it appears possible to reduce the incidence by two-thirds with antrypol alone. In Nigeria, on the other hand, results with suramin alone have been rather surprising. Twenty cases, all of them symptomless with one exception but having trypanosomes in the cervical lymph nodes, were given a single injection of 1 gm. of suramin. Trypanosomes disappeared from the lymph nodes during an observational period of thirteen months, but an examination at thirteen months showed that seven had abnormal findings in the cerebrospinal fluid : two patients with abnormal findings at seven months were normal at thirteen months and others showed improvement compared with seven months. A control series of twenty-one similar cases was given the standard course of suramin 3 gm. and tryparsamide 10 gm. without significant improvement in the results.

In practice, therefore, suramin is generally combined with tryparsamide or other arsenicals. Synergistic treatment was originally advised by Ehrlich many years ago and has been favoured by French workers, especially in chronic cases (Sicé, 1937).

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Aromatic Diamidine Derivatives

In experimental trypanosome infections the blood sugar is decreased and trypanosomes, unlike malaria parasites, rapidly die in the absence of glucose (Christophers and Fulton, 1938). Poindexter (1935) found that injections of insulin *in vivo* decrease the rate of multiplication of trypanosomes and prolong the life of the host, insulin shock being more readily produced than in normal animals. von Jancsó and von Jancsó (1935) and Schern and Artagaveytia-Allende (1935, 1936) then showed that synthalin and synthalin B had a therapeutic effect on *T. brucei* infections in mice and *T. equiperdum* infections in rats, the morphological changes in the trypanosomes being very similar to those produced by suramin. This suggested that synthalin and synthalin B acted directly on trypanosomes rather than indirectly through their action on the blood sugar. This view was confirmed by the experiments of Lourie and Yorke (1937), who found that at 37° C. *in vitro* synthalin exerts trypanocidal action within twenty-four hours in a dilution of 1 : 256,000,000. The action of synthalin on trypanosomes could thus not be directly associated with hypoglycæmia. Browning (1938) obtained similar results with *T. brucei* and *T. congolense.* Synthalin also acted on an acetarsol-resistant strain of *T. brucei* equally as well as on a normal strain.

The trypanocidal action of a number of guanidine derivatives was next studied by King *et al.* (1937) on the grounds that the chemical structure of synthalin differs essentially from that of all other trypanocidal substances. In a series of homologues of synthalin trypanocidal action *in vitro* rises as the number of methylene groups in the alkylene chain increases till, when the number of methylene groups is ten, the action is comparable to that of the tervalent arsenicals. Thus *in vitro* decamethylene diguanidine dihydrochloride (synthalin), dodecamethylene diguanidine dihydrochloride (synthalin B), and tetradecamethylene diguanidine dihydrochloride are all trypanocidal in a dilution of 1 in 256,000,000. Unfortunately, as the alkylene chain increases so does toxicity for animals. *In vivo*, therapeutic activity begins with the octamethylene member of the series, maximum tolerated doses of which clear the blood of infected mice for the space of five or six days. Alkylguanidines exhibit trypanocidal action *in vitro* but less than that of corresponding diguanidines; *in vivo* their action is nil. Certain simple cyclic guanidines also have a very weak action *in vitro*: this weak action is specially noteworthy in the case of diguanylspermine, which differs from synthalin only in the breaking of the chain of ten methylene groups by two imino groups. The guanidine grouping NH : C(NH_2)NH— is not, however, essential for trypanocidal activity, as is shown by the examination of a series of alkylene-di*iso*thioureas in which the connecting link between the amidine grouping NH : C(NH_2)— and the carbon (alkylene) chain is not an imino group —NH— but a sulphur atom, *e.g.*, NH : C(NH_2)—S—CH_2— : here also *in vitro* trypanocidal action increases as the series is ascended. High trypanocidal activity is present in the alkylene diamidines in which two amidine groups are attached directly to the ends of the methylene chain without the intervention of other groups or atoms. Again, maximum activity is reached as the series is ascended, undecane diamidine exhibiting pronounced trypanocidal activity *in vitro* and a curative action in infected mice and rabbits. Activity is decreased in the amidine series with members containing more than eleven carbon atoms.

When the amidine group is replaced by an amino group there is still a noticeable trypanocidal action *in vitro* which seems to reach a maximum in the hexadecylamine member of the series. In the alkylene-diamines a still greater activity is observed *in vitro* and possibly the maximum for the series has not yet been reached.

Since quaternary ammonium compounds possess only slight trypanocidal activity and cannot give rise to free bases by hydrolysis it is possible that the trypanocidal activity of guanidines, amidines, amines, and *iso*thioureas is a property of the free bases, since all these groups are strong bases forming neutral salts.

Aromatic Diamidines. Therapeutically in mice, King *et al.* (1937) found that with *T. rhodesiense* infections undecane diamidine appears to be superior to decamethylene diguanidine (synthalin) for while the maximum tolerated dose of synthalin is from 0.075 to 0·1 mgm. per 20 gm. mouse, the corresponding dose of undecane diamidine is at least 0·25 mgm. The minimum lethal dose of undecane diamidine is about 0·5 mgm. as compared with 0·15 to 0·25 mgm. for synthalin.

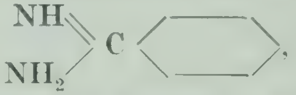
In the rabbit the maximum tolerated dose of undecane diamidine (10 mgm.per kgm.) causes a transient hyperglycæmia with nitrogen retention. Nausea is a prominent symptom (Devine, 1938). Undecane diamidine has little curative action on mice infected

The Trypanocidal Activity of the Alkylene Diamidines

Compound.	Trypanocidal titre *in vitro.*	Therapeutic effect.	Toxicity in gm. per 20 gm. mouse.
Octamethylenediamidine, 2 HCl .	1 : 8,000,000	Very slight	1·0
N-Decane 1 : 10-diamidine, 2 HCl .	1 : 64,000,000	Curative	0·5
N-Undecane 1 : 11-diamidine, 2 HCl	1 : 256,000,000	,,	0·5
N-Dodecane 1 : 12-diamidine, 2 HCl	1 : 64,000,000	,,	0·5

with *T. congolense,* but when given in large doses on the day of inoculation and on the nine following days it exhibits a prophylactic action. It has no curative action on *T. cruzi* infections in mice.

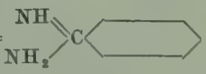
A number of aromatic amidine and guanidine compounds were found by King *et al.* (1938) to show a pronounced trypanocidal action *in vitro,* and with three of them, *pp'*-diguanidino-diphenylmethane, *pp'*-diamidino-diphenylmethane and 2 : 7-naphthalene diamidine, it was possible to cure mice infected with *T. rhodesiense.*

The trypanocidal activity of a series of aromatic compounds containing the amidine group, prepared by A. J. Ewins, was studied by Lourie and Yorke (1939) and Fulton and Yorke (1942). Preliminary investigations showed that in compounds where R, representing , is linked by a straight —C— linkage, by an —O— linkage, or by an —N— linkage, trypanocidal activity against *T. rhodesiense* is present : activity is also found in 2 : 7 diamido fluorene.

The results with the first series of compounds studied by Lourie and Yorke (1939) is shown in the table on page 417.

The most effective of these compounds were 4 : 4'-diamidino stilbene (stilbamidine), which has a chemotherapeutic index in mice against *T. rhodesiense* of about 30, 4 : 4'-diamidino diphenoxy propane (propamidine) and 4 : 4'-diamidino diphenoxy pentane (pentamidine) : the last two have chemotherapeutic indices of about 15. These compounds were at first used as the hydrochlorides, which are not nearly so soluble as the isethionates now available. Compounds $R—O(CH_2)_2O—R$ and

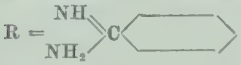
THE TRYPANOCIDAL ACTIVITY OF CERTAIN AROMATIC DIAMIDINES AGAINST *T. rhodesiense* (Lourie and Yorke, 1939)

Compound	Chemical name	MED.	MCD.	MTD.
		(mgm. per 20 gm. mouse).		
R = 				
4 : 4′—R—CH_2—R	4 : 4′-diamidino-diphenyl-methane.	0·1	1·0	1·0
4 : 4′—R—CH_2CH_2—R	4 : 4′-diamidino-diphenyl-ethane.	0·025	0·25/0·5	1·0
4 : 4′—R—$CH_2CH_2CH_2$—R	4 : 4′-diamidino-diphenyl-propane.	0·05 0·05	0·2 0·5	0·4 intraven. 1·6 subcutan.
4 : 4′—R—CH : CH—R	4 : 4′-diamidino stilbene	0·00625	0·025/0·05	1·0
4 : 4′—R—CH : CHCO—R	4 : 4′-diamidino benzalacetophenone.	Nil. Nil.	Nil. Nil.	1·0 intraven. 2·0 subcutan.
3 : 4′—R—CH : CH—R	3 : 4′-diamidino-stilbene	0·025	0·25/0·5	0·5
4 : 4′—R—O—R	4 : 4′-diamidino-diphenyl ether	0·025/0·05	0·25	0·5/1·0
4 : 4′—R—CH_2O—R	4 : 4′-diamidino phenyl benzyl ether	0·0125	0·1	1·0/2·0
3 : 4′—R—CH_2O CH_2—R	3 : 4′-diamidino phenyl benzyl ether.	0·125	0·25	1·0
4 : 4′—R—OCH_2OCH_2O—R	4 : 4′-diamidino-dibenzyl ether	Nil. Nil.	Nil. Nil.	0·4 intraven. 0·6 subcutan.
4 : 4′—R—$CH_2OC_6H_4O$ CH_2—R	1 : 4-di-(4′-amidinobenzyloxy) benzene.	— 0·8	Nil. 1·2	0·2 intraven. 2·4 subcutan.
4 : 4′—R—$OCH_2C_6H_4CH_2O$—R	ω : ω′di-(*p*-amidino-phenoxy)-xylene.	0·1	Nil. 1·2	0·2 intraven. 2·4 subcutan.
4 : 4′—R—CH_2CH_2O—R	4 : 4′-diamidino-β-phenyl ethyl ether.	0·05 0·05	0·3 0·8	0·3 intraven. 1·2 subcutan.
4 : 4′—R—OCH_2O—R	4 : 4′-diamidino-diphenoxy methane.	0·025/0·05	0·25/0·5	0·5
4 : 4′—R—$O(CH_2)_2O$—R	4 : 4′-diamidino-diphenoxy-ethane.	0·025	0·1	1·0
4 : 4′—R—$O(CH_2)_3O$—R	4 : 4′-diamidino-diphenoxy-propane.	0·0125	0·05/0·1	0·5/1·0
3 : 3′—R—$O(CH_2)_3O$—R	3 : 3′-diamidino-diphenoxy-propane.	0·025	0·5	1·0
4 : 4′—R—$O(CH_2)_5O$—R	4 : 4′-diamidino-diphenoxy-pentane.	0·01	0·05/0·1	1·0
3 : 3′—R—$O(CH_2)_5O$—R	3 : 3′-diamidino-diphenoxy-pentane.	0·1 0·1	Nil. 0·4	0·5 intraven. 1·6 subcutan.
4 : 4′—R—$O(CH_2)_{10}O$—R	4 : 4′-diamidino-diphenoxy-decane.	0·05 0·05	Nil. 0·5	0·8 intraven. 3·0 subcutan.
4 : 4′—R—$O(CH_2)_2NH$—R	4 : 4′-diamidino-β-phenoxy-ethyl aniline.	0·025	0·1/0·25	1·0
4 : 4′—R—NHCONH—R	4 : 4′-diamidino-diphenyl urea	0·1 0·4	0·4 0·8	0·4 intraven. 1·2 subcutan.

The diamidines were used in the form of their dihydrochlorides.
MED. = Minumum effective dose.
MCD. = Minimum curative dose.
MTD. = Maximum tolerated dose.

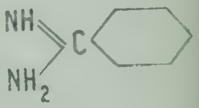
R—OCH_2O—R are only slightly less active and these are followed by R—$O(CH_2)_2NH$—R. In the series R—$O(CH_2)_nO$—R trypanocidal activity increases with length of the alkane chain to a point

AROMATIC DIAMIDINES: *T. congolense* AND *T. rhodesiense* INFECTIONS IN MICE, (20 GM.) INTRAPERITONEAL INJECTIONS
(Fulton and Yorke, 1942)

Compound.			MED.	MCD.	MTD.
					
$4:4'\text{—}R.C⋮C.R.$	4 : 4′-diamidino-tolane	*T. rhodesiense*	0·01	0·05/0·1	0·5
		T. congolense	0·5	—	0·5
$4:4'\text{—}R.\overset{H_3C}{C}:\overset{CH_3}{C}.R$	4 : 4′-diamidino-dimethyl stilbene.	*T. rhodesiense*	0·025	—	1·0
		T. congolense	0·1	0·25/0·5	1·0
$4:4'\text{—}R.\overset{H_3C}{C}:CH.R$	4 : 4′-diamidino-monomethyl stilbene.	*T. rhodesiense*	0·01	0·05	1·0
		T. congolense	0·5	1·0	1·0
$4:4'\text{—}R.(CH_2)_6.R$	4 : 4′-diamidino-diphenyl hexane.	*T. rhodesiense*	0·025	—	0·25
		T. congolense	—	—	0·25
$4:4'\text{—}\overset{OH}{R}.CH:CH.R$	4 : 4′-diamidino-2-hydroxy-stilbene.	*T. rhodesiense*	0·005	0·05	1·0
		T. congolense	0·25	—	1·0
$4:4'\text{—}R.NH.CO.NH.R$	4 : 4′-diamidino-diphenyl urea.	*T. rhodesiense*	0·05	0·25	0·5
		T. congolense	0·25	—	0·5
$4:4'\text{—}R.SO_2.R$	4 : 4′-diamidino-diphenyl sulphone.	*T. rhodesiense*	—	—	1·0
		T. congolense	—	—	1·0

All diamidines were in the form of hydrochlorides.
MED. = Minimum effective dose.
MCD. = Minimum curative dose.
MTD. = Maximum tolerated dose.

when $n = 5$; beyond this activity decreases and when $n = 1$ is reached there is hardly any activity.

Comparatively slight changes in the radical  completely destroy trypanocidal activity *in vivo.*

The toxicity of 4 : 4′-diamidino monomethyl stilbene for mice varies considerably according to the route of administration, but always the therapeutic indices $\frac{MTD}{MED}$ and $\frac{MTD}{MCD}$ were most effective when the drug was given by the subcutaneous route. The figures are given in the table on page 419.

The disadvantage of the subcutaneous route for stilbamidine is that in mice it gives rise to ulcers.

Tests on rabbits in an advanced stage of infection due to *T. rhodesiense* showed that while the maximum tolerated dose of

THE TOXICITY AND THERAPEUTIC ACTION OF 4 : 4′-DIAMIDINO MONOMETHYL STILBENE (MGM. PER 20 GM. MOUSE) IN MICE INFECTED WITH *T. rhodesiense*. (Fulton and Yorke, 1943a.)

Route of administration.	MTD.	MED.	MCD.	$\frac{\text{MTD.}}{\text{MED.}}$	$\frac{\text{MTD.}}{\text{MCD.}}$
Oral . .	8	0·25	1	32	8
Subcutaneous.	2	0·01	0·025/0·05	200	80/40
Intraperitoneal	1	0·01	0·05	100	20
Intravenous .	0·25	0·005	0·025	50	10

MTD. = Maximum tolerated dose.
MED. = Minimum effective dose.
MCD. = Minimum curative dose.

stilbamidine is about 20 mgm. per kgm., single doses of 1·25 to 5 mgm. per kgm. of body weight cured most animals and with small repeated doses of 0·5 mgm. repeated on each of five consecutive days the results were even better.

Propamidine and pentamidine also were curative in rabbits infected with *T. rhodesiense*. Infections due to *T. cruzi* in mice were uninfluenced. With *T. congolense* infections in mice results were less satisfactory and cures were obtained only with doses approaching the maximum tolerated. Stilbamidine was active, especially with repeated doses of 0·25 mgm. given on each of three consecutive days, but 4 : 4′-diamidino-α, β-dimethyl stilbene was even more effective in laboratory animals (Fulton and Yorke, 1943a and b).

The toxicity and therapeutic efficiency of the dimethyl stilbene in the treatment of *T. congolense* infections in mice was investigated by Fulton and Yorke (1943b). The MTD. by the various routes. is very similar to that for the monomethyl derivatives. Apart from the oral route, the values for MED. and MCD. in the case of the other routes are almost identical. Each, however, is much larger than the corresponding dose used in the treatment of *T. rhodesiense* by the monomethyl derivative, and this fact is reflected in the smaller values for the indices $\frac{\text{MTD}}{\text{MED}}$. and $\frac{\text{MTD}}{\text{MCD}}$. The length of time required to make the blood negative by the MED.

and MCD. is greater than in the case of *T. rhodesiense* infections. Intravenous administration causes collapse even with the MCD., while subcutaneous injection produces superficial ulceration.

The toxicity and therapeutic action of 4 : 4′-diamidino dimethyl stilbene in mice infected with *T. congolense* is shown in the table :—

The Toxicity and Therapeutic Action of 4 : 4′-diamidino Dimethyl Stilbene (mgm. per 20 gm. Mouse) in Mice Infected with *T. congolense* (Fulton and Yorke, 1943a).

Route.	MTD.	MED.	MCD.	$\frac{\text{MTD.}}{\text{MED.}}$	$\frac{\text{MTD.}}{\text{MCD.}}$
Oral	8	8	16	1	<1
Subcutaneous	1	0·01	0·25	100	4
Intraperitoneal	0·5	0·025	0·5	20	1
Intravenous	0·1	0·025	0·25	4	<1

MTD. = Maximum tolerated dose.
MED. = Minimum effective dose.
MCD. = Minimum curative dose.

Fulton and Yorke (1943b) compared the efficiency against *T. congolense* infections in mice of 4 : 4′-diamidino dimethyl stilbene, 4 : 4′-diamidino monomethyl stilbene, 4 : 4′-diamidino stilbene, tartar emetic, and 7-amino-9(*p*-aminophenyl)-10 methyl phenanthridinium chloride. The last has a wider effective range than the diamidines (*cf.* p. 437).

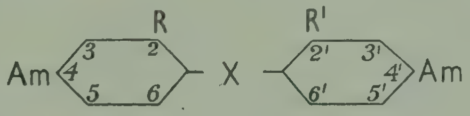
Wien (1946) also studied the toxicity of 4 : 4′-diamidino-α : β-dimethyl-stilbene dihydrochloride. By intravenous injection into mice the LD 50 was 0·49 mgm. per kgm., but subcutaneously the LD 50 was 0·198 mgm. per kgm. Its action on strains of *T. congolense* varied somewhat from strain to strain as shown in the table :—

Strain.	CD 50 (mgm./kgm.).	Limits % (p = ·95).	Therapeutic index.
I	0·0057	58–171	35
II	0·0041	63–160	49
III	0·0162	60–167	12
IV	0·020	61–163	10
V	0·0078	61–164	25

An attempt was also made to correlate chemical constitution with activity against *T. congolense.* The introduction of methyl groupings into the unsaturated stilbene linkage enhanced activity but nuclear substitution was not so effective. Nuclear substitution by various groupings in the 2-position did not increase activity except for a slight effect with methyl, amino, and acetamido derivatives, though the introduction in the 2-position of halogens Cl, Br, and I, or Me, and OH, increased activity against *T. equiperdum* or *T. rhodesiense.* Alkyl substitution in the amidine grouping and substitution of a diphenylamine for the stilbene linkage were without effect. Phenanthrene-3 : 6-diamidine was of high activity, but an analogous compound 3 : 6-diamidinocarbazole had no trypanocidal action. The toxicity and activity of diamidine compounds examined by Wien (1946) against *T. congolense* are shown in the table :—

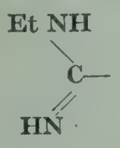
THE TOXICITY AND ACTIVITY OF DIAMIDINE COMPOUNDS AGAINST *T. congolense* (Wien, 1946)

(1) Stilbene series

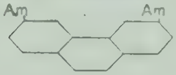
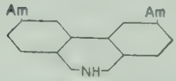


Substituent groups.			LD 50 (mgm./kgm.).	ED 50 (mgm./kgm.).	CD 50 (mgm./kgm.).	Index.
X	R	R'				
—CH : CH—	H	H	0·18	0·02	0·08	2·3
,,	Me	H	0·146	0·01	0·03	4·9
,,	Me	Me	0·140	0·015	Not curative	—
,,	OCH_3	H	0·100	0·015	,, ,,	—
,,	OH	H	0·137	0·025	,, ,,	—
*,,	OH	OH	0·095	Inactive	—	—
,,	I	H	0·332	0·025	Not curative	—
,,	NH_2	H	0·075	0·015	0·038	2·0
,,	NH . Ac	H	0·150	0·008	0·038	4·0
**,,	H	H	0·180	0·04	Not curative	—

* Am = 5 : 5′. ** Am =



(2) Stilbene and diphenoxy series

Substituent groups.			LD 50 (mgm./kgm.).	ED 50 (mgm./kgm.).	CD 50 (mgm./kgm.).	Index.
X	R	R′				
—C(Me) : CH—	H	H	0·12	0·01	0·04	3·0
—C(Me) : C(Me)—	H	H	0·198	0·004	0·007–0·016	12–28
—NH—	H	H	0·050	0·01	Not curative	—
—O—$(CH_2)_3$—O	H	H	0·055	Inactive	—	—
,,	Br	H	0·107	0·01	0·05	2·1
—O—$(CH_2)_5$—O	Br	H	0·23	Inactive	—	—
—O—$(CH_2)_7$—O	H	H	0·063	,,	—	—
			0·160	—	<0·01	16
			0·065	0·01	Not curative	

LD 50 = average lethal dose.
ED 50 = (50 per cent. of animals cleared of trypanosomes from the peripheral blood within seven days).
CD 50 = (50 per cent. of animals free from trypanosomes for at least twenty-eight days).

$$\text{Chemotherapeutic index} = \frac{\text{LD 50}}{\text{CD 50}}.$$

A series of monoamidines was examined by Goodwin and Marshall (1945) but all were without action on either *T. equiperdum* or *T. cruzi.*

The action of diamidines on *T. equinum* has received comparatively little attention. Launoy and Chaboud (1948), however, showed that in mice pentamidine in doses of 0·1 mgm. subcutaneously cured 30 per cent. and 0·2 mgm. cured 100 per cent. of animals. In the rat a single dose of 1 mgm. cured 50 per cent. and 3 mgm. cured 100 per cent. ; in guinea pigs 1·75 to 2·0 mgm. per 100 gm. of body weight cured 100 per cent. of animals. The pharmacology of the diamidines which have proved active in protozoal infections is discussed on pp. 315–321.

A search has been made for other therapeutically active amidines. Barber and Slack (1947) found that neither tetrakis-*p*-amidinophenylethylene nor 5-cyano-3 : 3′ : 5′-triamidino-α γ-diphenoxy propane had any trypanocidal action against *T. equiperdum* in mice while they were more toxic for mice than the aromatic diamidines, presumably owing to the increased number of amidine groups in the molecule. Barber *et al.* (1947) also prepared phenanthridines with the therapeutically active amidine group: 3-amidino-9-methyl-phenanthridine and 3-amidino-9-*p*-amidino-phenyl-phenanthridine had little or no trypanocidal action: 7-amidino-9-*p*-phenylphenanthridine had a slight action against *T. equiperdum* but the methochloride of this base was quite inactive. Similarly according to Gregory *et al.* (1947) the pyridine analogues of propamidine and pentamidine, α(-bis-(5-amidino-2-pyridyloxy) pentane) dihydrochloride and 2-*p*-amidinostyrylpyridine had no trypanocidal activity: the latter, however, inhibited *Staphylococcus aureus* at a dilution of 1 : 64,000, less than half the active dilution of the carbocyclic analogue pentamidine.

T. congolense **Infections in Cattle.** 4 : 4′-Diamidino dimethyl stilbene was used by Carmichael and Bell (1943) in cattle infected with *T. congolense*: doses of 2·5 and 5·0 mgm. per kgm. of body weight given intravenously were unable to produce a cure: a dose of 10 mgm. per kgm. cured four cows but one relapsed and one was poisoned: with a dose of 12·5 mgm. there were two cures and two fatal cases of poisoning. With the same dose of 12·5 mgm. of body weight cure was produced in two cows, whereas a dose of 10 mgm. per kgm. was apparently ineffective. In those animals which exhibited fatal poisoning the symptoms, except in one instance, came on rapidly after the injection. The respiratory rate was increased, dyspnœa, profuse salivation, and death followed. No delayed poisoning was seen as with pentamidine. It is obvious that the therapeutic index in cattle is very small. Daubney and Hudson (1941) employed pentamidine in cattle with *T. congolense* infections, in addition to stilbamidine. Neither drug completely cured the infection. Pentamidine, when given in a single dose of 10 mgm. per kgm. of body weight, gave rise to immediate symptoms of poisoning, and repeated doses of 5 mgm. per kgm. of body weight caused toxic changes with fatty degeneration of the liver, usually after a delay of twenty-four days.

Treatment of Human Trypanosomiasis with Aromatic Diamidines

Stilbamidine, pentamidine, and propamidine have all been employed in the treatment of human trypanosomiasis in Africa.

Stilbamidine. The earliest investigations were carried out in the Gambia by Bowesman (1940), and in Northern Nigeria by McLetchie (1940) and Harding (1940). Bowesman gave intramuscular doses of 1 mgm. per kgm. twice a week. In five out of seven patients with 0·03 per cent. or more of protein (Ravaux albuminodosometer) in the cerebrospinal fluid there was an apparent cure; in two the result was doubtful: in thirteen others with the same amount of protein ten were well a year later. It was concluded that stilbamidine was suitable for the treatment of early and intermediate stages but quite unsuitable for patients with more than 0·05 per cent. of protein in the cerebrospinal fluid. McLetchie (1940) gave stilbamidine to fourteen cases of sleeping sickness: trypanosomes disappeared from the lymph nodes after from one to three injections but the effects on the cerebrospinal fluid could not be judged. Of thirteen cases treated by Harding (1940) three with mild or moderately severe symptoms were clinically cured and one was improved, but in no case did a pathological cerebrospinal fluid return to normal: in fact eight moderate or severe cases became worse. Apart from any question of toxicity, stilbamidine may therefore be regarded as of possible value in very early cases, but in later cases as of less value than a combined course of suramin and tryparsamide. Lourie (1942) regarded stilbamidine as of less value than pentamidine or propamidine. Doses of 0·5 mgm. per kgm. of body weight apparently give as good results as 1 mgm.

Pentamidine was tested by Saunders (1941) in fourteen patients with sleeping sickness in the Gold Coast. The dose was 1 mgm. per kgm. of body weight. Up to sixty-five injections were given without ill results. Trypanosomes were removed from the blood stream after four injections. In 1944 Saunders, Holden, and Hughes reported on some of the previously treated cases and on twelve additional patients given pentamidine. Patients with a cell count of less than 30 per c.mm. in the cerebrospinal fluid were usually cured, but when the number of cells was over this figure a relapse generally occurred. In very advanced cases the drug was quite useless. Similar results were obtained by other observers. Lawson (1942) treated fifty-three unselected cases due to *T. gambiense* in the West Nile district of Uganda. Although the blood and lymph node juice were rapidly sterilised and the cell count in the cerebrospinal fluid was reduced it was agreed that tryparsamide was the better drug in advanced cases. Lourie (1942) gave eighty patients in Sierra Leone 100 mgm. of pentamidine daily for twelve days. In early cases pentamidine was the equal of tryparsamide but in late cases was useless. Gilbert (1943) gave doses of 2 mgm. per kgm. intravenously to fourteen patients but found that such amounts caused headache and severe rigors : the cell count in the cerebrospinal fluid was reduced, but in two cases pentamidine failed and tryparsamide subsequently caused rapid clinical improvement. Intravenous medication would seem to be preferable to intramuscular injection.

More recent observations on pentamidine (van Hoof *et al.*, 1944 ; van Hoof, 1947 ; Lester, 1945 ; McLetchie, 1948 ; Harding and Hutchinson, 1948 ; Saleun and Chassain, 1948) have amply confirmed the earlier conclusions. Pentamidine is of considerable value in early cases but of no value in those showing involvement of the central nervous system. The relapse rate in first-stage cases is not high. Ceccaldi *et al.* (1949) found that of twenty-two patients given 1·5 mgm. per kgm. on the first day, 1·75 mgm. on the second day, and 2·0 mgm. per kgm. on the third, fourth and fifth days, six had developed nervous symptoms in from sixteen to twenty-four months. It has a strong trypanocidal action on *T. gambiense* whether or not the strain is arsenic-resistant. Trypanocidal action is, however, somewhat slow in starting and is observable only on the third day after an optimal daily dosage of 1 mgm. per kgm. of body weight. To avoid toxic effects, doses of 2 mgm. per kgm. and over must not be repeated more than twice a week. Repeated doses increase the curative action as a result of the accumulation of the drug in the body.

Intramuscular injections are less toxic and quite as effective as intravenous inoculations. Infected tsetses fed on animals injected with therapeutic doses of pentamidine are not thereby rendered free from trypanosomes nor is the cyclical development of the trypanosomes influenced in any way.

There is now evidence that pentamidine may be useful if given together with tryparsamide in intensive courses for seven to ten days. Such courses involve the administration of 1·3 gm. of pentamidine and 6 gm. of tryparsamide in seven to ten days :

such a combination is suitable when the number of cells per c.mm. is less than ten: with higher cell counts the tryparsamide dosage is increased. In twenty-six cases thus treated the results were equal to those obtained in twenty-four cases with suramin 3·2 gm. and tryparsamide 6 gm. in the same period. Seven months later all patients were well and most of the young adult females were pregnant, an almost infallible sign that the infection has been eliminated. Harding (1945), and McLetchie (1948) find the combination non-toxic, as do Trinquier and Arnoult (1947). Ceccaldi and Arnoult (1949) successfully treated two patients in the first stage who had relapsed after a course of orsanine: both patients were alive and well thirty-six and thirty-eight months later. Another patient who harboured an arsenic-resistant trypanosome was given pentamidine intravenously and was in good health thirteen months later.

In French West Africa pentamidine is being employed intramuscularly and intrathecally, despite its toxicity, in very late stage patients, many of whom die in a short time but some of whom have undoubtedly been improved as judged by several months' observation. Pentamidine is preferred to suramin, as treatment can be completed in ten days as compared with five weeks with the latter compound (Nodenot, 1946).

The prophylactic action of pentamidine is referred to on p. 465.

Propamidine has been less extensively used in the treatment of trypanosomiasis than pentamidine. Lourie (1942), in Sierra Leone, believed that in early cases propamidine was equal to tryparsamide but much less effective in late cases. van Hoof, Henrard and Peel (1947) also used propamidine in the treatment of early cases with success: five early cases were given 1 to 2 mgm. per kgm. daily, the total dose never exceeding 25 mgm. per kgm. Secondary cases with raised albumin-content in the cerebrospinal fluid did not show any decrease in protein content, and in some instances an increase.

Toxic Reactions. Stilbamidine has been reported to produce a number of toxic reactions in patients suffering from trypanosomiasis. These reactions include a sudden fall in blood pressure, vomiting, and salivation with nausea: these symptoms are most common after the first few injections. A rapid pulse, sweating, dizziness, faintness, loss of consciousness, epileptiform twitching, and incontinence of urine and fæces may occur. Recovery usually occurs after some thirty minutes. Stilbamidine does not cause eye symptoms or albuminuria and is non-irritant when given intramuscularly. Pentamidine is less toxic. Itching, according to Saunders (1941), was very common. A curious puffy suffusion of the face and eyelids was often seen but it usually lasts for only a few minutes. Intramuscular are usually less toxic than intravenous injections. McComas and Martin (1944) described a fatal case in an African soldier who had received three intravenous injections of 100 mgm. of pentamidine. Ten minutes after the last injection the soldier passed into status epilepticus. His temperature rose and remained between 102° and 103° F. till death occurred about a fortnight later. Suggestions have been made that pentamidine in therapeutic doses may tend to cause abortion, but these have not been confirmed. Propamidine is generally regarded as being less toxic than stilbamidine but there is insufficient evidence to compare it accurately with pentamidine when administered to patients with sleeping sickness. van Hoof, Henrard and Peel (1947), from observations on a few cases, believe that propamidine is more toxic than pentamidine, since paresis and paralysis has occurred during treatment, thus suggesting some action on the anterior horn cells of the cord.

Guanidine Derivatives

King *et al.* (1937) originally showed that guanidine compounds possess some action in experimental trypanosomiasis. Activity was then found in alkylene diamidines in which two guanyl groups are attached to the ends of the methylene chain without the intervention of other groups. In view, however, of the pronounced action of the aromatic diamidines little further interest was taken in guanidines until Hewitt *et al.* (1949) reinvestigated their trypanocidal activities. It was found that *p*-phenylene diguanidine and certain related derivatives were active against *T. equiperdum* in mice, although less effective when administered parenterally than arsenicals, suramin or stilbamidine. These compounds are active when given in multiple doses orally. The comparative effectiveness of four guanidine derivatives is shown in the table. Of the guanidines tested none showed greater activity or were better tolerated than *p*-phenylene diguanidine.

COMPARISON OF THE EFFECT OF GUANIDINE DERIVATIVES WITH STILBAMIDINE AND SURAMIN AGAINST *T. equiperdum* IN MICE (Hewitt *et al.*, 1949)

Compound.	Approximate suppressive dose (minimum therapeutic dose), mgm. per kgm. twice daily for 3½ days.		Approximate curative dose (CD 50), mgm. per kgm. twice daily for 3½ days.		Ratio of intraperitoneal to oral treatment.	
	I.P.	Oral	I.P.	Oral	I.P.	Oral
p-Phenylene diguanidine	2·0	10·0	4·0	20·0	1 : 5	1 : 5
1, 3-Bis-(4-guanidophenyl) guanidine tri HCl	1·0	45·0	2·0	60·0	1 : 45	1 : 30
Bis-(4-guanidophenyl)-sulphide H_2CO_3	1·0	10·0	2·0	20·0	1 : 10	1 : 10
3, 3′-Dimethyl-4, 4′-diguanido biphenyl	1·0	20·0	2·0	60·0	1 : 20	1 : 30
Stilbamidine	0·0175	1·6	0·025	3·0	1 : 91	1 : 120
Suramin	0.15	50·0	0·2	100·0	1 : 333	1 : 500

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Derivatives of Ethylene Diamine

Funke and Fourneau (1942) suggested that diamines derived from benzylamine possessed some trypanocidal action. The question was further studied by Funke and his colleagues (1943) who found that eight of thirty-nine different compounds possessed some trypanocidal activity when injected intravenously or given by mouth to mice infected with *T. brucei*. The eight compounds, which were sufficiently active to cure three-quarters of the infected mice, were :—

2440 R.P.	(*p*-ethylbenzylamino)-1 amino-2 ethane $(C_2H_5 \,.\, C_6H_4 \,.\, CH_2 \,.\, NH \,.\, CH_2 \,.\, CH_2 \,.\, NH_2)$
1986 F	(*p*-*n*-propylbenzylamino)-1 amino-2 ethane
1921 F	(*p*-*iso*propylbenzylamino)-1 amino-2 ethane

and the corresponding (*p*-sec. butyl-benzylamino)-, β-tetrahydro-menaphthylamino-, *iso*propyl-menaphthylamino-, methyl-2-*iso*-propyl-5-benzylamino-, and chloro-4-benzylamino- derivatives. Compound 1921 F, the most active of the group, was as active against *T. brucei* in guinea pigs as in mice. Trypanocidal activity seems to depend on the presence of a diamine structure in the chain and on a second group, such as $—C_2H_5$, substituted in the benzene ring. Possibly, however, the presence of a benzene ring is not necessary for trypanocidal action. No tests have yet been carried out on man with this group of compounds, but Lwoff *et al.* (1944) investigated their activity against a non-pathogenic flagellate, *Strigomonas oncopelti*, and in addition found that N-*p*-*iso*propylbenzyl ethylene diamine had some action on *T. cruzi* : they form, however, a new group of trypanocidal drugs, differing completely in structure from other known chemical types.

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Quinoline Derivatives

Anil- and Styryl-Quinoline Derivatives. Derivatives of anil- and styryl-quinolines were introduced in the treatment of trypanosomiasis by Browning, Cohen, Ellingworth, and Gulbransen (1926 and 1929). The existence of trypanocidal activity in these compounds appears to depend on the presence in each of the nuclei of basic groups or at any rate of acylamino groups. The most marked trypanocidal action is exerted by substances which contain a free basic group in one nucleus and an acylamino group in the other. This was especially noticeable in the styryl series, which were, as a rule, much more effective than the anils as trypanocidal agents. The two most active compounds prepared were 2(*p*-acetylaminostyryl)-6-dimethylaminoquinoline methochloride and 2(*p*-aminostyryl)-6-acetylaminoquinoline methochloride. Further anologues of these anil and styryl derivatives were examined by Browning, Cohen, Cooper, and Gulbransen (1932). Compounds endowed with trypanocidal activity are 2(*p*-aminostyryl)-6-acetyllactylaminoquinoline methochloride, 2(*p*-aminostyryl)-6-lactylaminoquinoline methochloride and also 2(*p*-aminostyryl)-6-glycerylaminoquinoline methochloride, though on testing a series of doses the range of action appeared to be less than in the compound 2(*p*-aminostyryl)-6-acetylaminoquinoline methochloride of which they are analogues. 2(*p*-lactylaminostyryl)-6-aminoquinoline methochloride had a wide range, and by acetylation of the amino group in the quinoline nucleus trypanocidal activity was not greatly altered. Browning, Cohen, Ellingworth, and Gulbransen (1931) also studied the trypanocidal activity of a number of derivatives of benzthiazole in which the pyridine ring of the quinoline group is replaced by a five-membered ring containing nitrogen and sulphur. Both anil and styrylbenzthiazole derivatives exhibited trypanocidal action when the appropriate groups were present in the respective nuclei, when, that is to say, as in the quinoline series, one nucleus contained a basic group, the other an acetylamino group. As in the quinoline series, compounds containing the following constituent groups were possessed of trypanocidal action :—

Styryl No.	Group in benzene nucleus.	Group in benzthiazole nucleus.
332	NH_2	$NH . COCH_3$
349	$NH . CH_3$	$NH . COCH_3$
330	$N(CH_3)_2$	$NH . COCH_3$
368	$NH . COCH_3$	NH_2
333	$NH . COCH_3$	$NH_2 . COCH_3$
377	$NH . COCH_3$	$N(CH_3)_2$

In the anilbenzthiazole group some action was observed in all the compounds prepared except with 2(*p*-dimethylaminoanil) benzthiazole methochloride, but the only compound producing cure was 2(*p*-methylaminoanil) acetylaminobenzthiazole methosulphate.

A further series of anil and styryl derivatives of 4-aminoquinaldine was investigated for trypanocidal action by Ashley, Browning, Cohen and Gulbransen (1933). In these compounds the substituent group in the quinoline nucleus is situated in the 4-position and consists of a primary, tertiary or acetylated amino group. 2(*p*-Dimethylaminostyryl)-4-acetylaminoquinoline methochloride was without action, whereas the 6-analogue, although only slightly less toxic for the mammalian host, is an effective trypanocidal agent. 2(*p*-Dimethylaminoanil)-4-acetylaminoquinoline methochloride occasionally produced a cure, whereas the 6-analogue, which is three times more toxic, has only a slight trypanocidal action.

A further series in which the substituent groups in the quinoline nucleus, either primary, tertiary or acetylated *para*-aminobenzoylamino, are in the 6-position has been investigated by Browning, Cohen, Cooper, Ellingworth, and Gulbransen (1933). In the anil derivatives there was some trypanocidal action but no cure was produced. Of the styryl compounds those with a dimethylamino group in the benzene nucleus were without trypanocidal action, but others were active. Thus 2(*p*-acetylaminostyryl)-6(*p*-aminobenzoylamino) quinoline methoacetate had a chemotherapeutic index of 1 : 240 : 2(*p*-acetylaminostyryl-6(*p*-dimethylaminobenzoylaminoquinoline methoacetate) did not act in so wide a range of dosage ; certain others were rather irregular in their action. Styryl-quinoline derivatives are not without toxicity : N-2-(*p*-diethylaminostyryl)-6-methoxyquinoline ethochloride and possibly some others may cause necrosis in the cells of the islands of Langerhans and in the convoluted tubules of the kidney (Dunn *et al.*, 1943 ; Lukens and Kennedy, 1949).

Surfen C

Of a large number of derivatives of 4-aminoquinoline, one compound, bis-2-methyl-4-aminoquinolyl-6-melamine, " surfen C " (Iensch, 1937), has been used in the treatment of trypanosome infections. Surfen C must be distinguished from " surfen," bis-2-methyl-4-aminoquinolyl-6-carbamide, which has recently been introduced as a non-staining antiseptic and is claimed to be more active in living tissues than *in vitro* (von Schürer and Homma, 1937). Although pathogenic trypanosomes isolated from man are curable when maintained in mice, the same strains in human beings are not so readily affected and, in addition, surfen C has a toxic action on the kidney. In mice, infections due to *T. brucei* and *T. congolense* are readily cured but, according to Lester (1935), in Nigeria three doses at weekly intervals of 10 mgm. per kgm. of body weight do not prevent relapses in sheep infected with *T. congolense* and *T. vivax*, although the therapeutic results are better than with tartar emetic. In Nigeria also, cattle infected with these trypanosomes are not cured by three intramuscular injections of 10 mgm. per kgm. of body weight, as was the case in Uganda with cattle infected by *T. brucei* and *T. congolense.* Six months later, however, all the Nigerian cattle treated by surfen C were alive, whereas only 6·2 per cent. of controls survived for the same period. Evans (1936) found that in cattle infected with *T. congolense* a single dose of surfen C was equal in effect to five injections of antimosan. Good results were also obtained in the Belgian Congo by van Saceghem (1938) and Delidimitriou (1938). Stewart (1937), on the Gold Coast, found that surfen C was of little value in cattle, but curative in canine trypanosomiasis.

In Northern Rhodesia the results in cattle were disastrous: some animals died within fifteen minutes of the injection with froth and blood running from the nostrils, and at post-mortem hæmorrhages were present in the lungs (Le Roux, 1937). van Rensburg (1938), in South Africa, also obtained unfavourable results.

In horses infected in the Sudan with *T. congolense*, Bennett (1936) found that almost all animals could be cured with surfen C, though the amount of the drug necessary to ensure a cure varied. Some horses were cured by a single dose of 1 gm. per 100 kgm. of body weight. Horses should not be given surfen C intravenously, and on subcutaneous administration large swellings develop at the point of injection: occasionally the same trouble follows intramuscular administration. No generalised toxic reactions were seen in horses.

In most areas surfen C has now been discarded for cattle owing to the toxic results following both intramuscular and intravenous injection.

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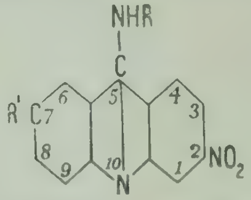
Acridine Derivatives

Acriflavine which has long been known to possess trypanocidal properties is, according to the British Pharmacopœia, Addendum

(1936), a mixture of 2 : 8-diamino-10-methylacridinum chloride and 2 : 8-diaminoacridine, and contains approximately one-third of its weight in the form of the latter compound.

Schnitzer and Silberstein (1929) found that in the case of the 6-nitro-9-aminoacridines the presence of the nitro group in position 6 enhances trypanocidal action and a further favourable effect is brought about by the substitution of a doubly alkylated amino group for a hydrogen atom in the amino group in position 5.



In this formula the NHR in position 5 is such a substituent group as the dimethylaminooxypropylamino group $NH . CH_2 . CHOH . CH_2 . NMe_2$, whilst R′ in position 7 is an alkyloxy group such as methoxyl (OMe). So far as trypanocidal action is concerned, the doubly alkylated basic side-chain (R) may either replace a hydrogen atom in the amino group at position 5 or may be directly attached to the carbon atom in position 6. Within a group of substances in which R has the same composition and position, modification of trypanocidal action could be brought about in most cases by variation of R′ (position 7); thus a 2-ethoxy compound is usually less active than a 2-methoxy compound and the latter less active than one in which there is no side-chain in position 7.

Toxicity and therapeutic effect do not always run on parallel lines in these compounds. In some groups the most toxic members are also the most effective therapeutically, in others only the possibility of large dosage allows the exhibition of any therapeutic activity. By variation of the side-chains within the limits described, Schnitzer and Silberstein believe, however, that it is possible to obtain a favourable adjustment between toxic action and therapeutic efficiency. Thus compound 3043a with a glycyldiethylaminoethylamino group, probably

$$CH_2 . NH_2 . CO . CH_2 . CH_2 . NEt . CH_2 . CH_2 . NH$$

in position 5 and an ethoxyl group (OC_2H_5) in position 7, has a chemotherapeutic index of from 1 : 20 to 1 : 50 and is, moreover, one of the few instances where an ethoxyl is more efficient than a methoxyl group in position 7.

The action of a number of pyrrole dyes has been tested by Fischl (1935) on *T. brucei* infections in mice. Pyrrol blue, pyrrol red, and phthalocyanin had no trypanocidal action.

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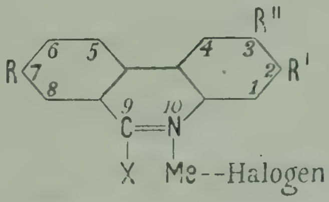
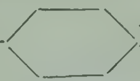
Phenanthridinium Compounds

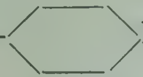
Two of a series of phenanthridine and phenanthridinium compounds were found by Browning *et al.* (1938) to possess curative action in mice experimentally infected with trypanosomes. The phenanthridinium derivatives which possess certain antiseptic properties (Morgan *et al.*, 1938) have the general formula



The compounds first investigated were

7-amino-9(p-aminophenyl)-10-methylphenanthridinium chloride (897) ($R = NH_2$; $R' = R'' = H$; X =—NH_2) and

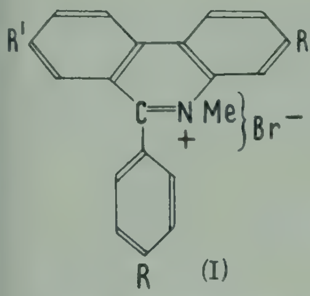
3(acetylamino) - 9(p - acetylaminophenyl) - 10-methyl-phenanthridinium chloride

($R'' = NH . COCH_3$; X =—$NH . COCH_3$).

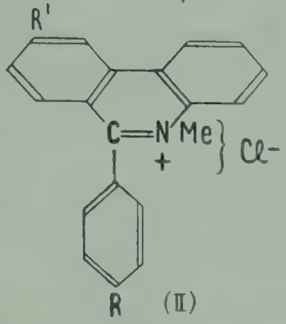
Both these substances, in doses approaching the maximum tolerated (one subcutaneous injection of 1 mgm. of 897 or 2·5 to

5·0 mgm. of the second compound for a mouse of 20 gm.), are curative for mice infected with *T. brucei* injected twenty-four hours before treatment. Compound 897 has a curative action also in *T. congolense* infections in mice and is thus of considerable interest since *T. congolense* is refractory to many drugs which are actively trypanocidal for *T. brucei.* The second compound has no action on *T. congolense* but both will inhibit the development of a strain of *T. brucei* completely resistant to acetarsol. The second compound also has a marked effect on *Spirillum minus* infections in mice though it does not lead to complete cure.

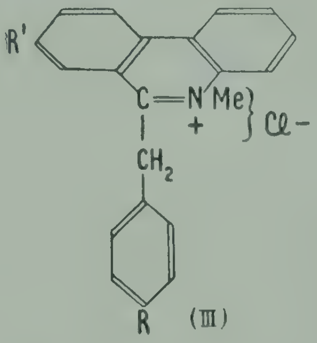
Against monomorphic trypanosomes and *T. cruzi* many *N*-heterocyclic compounds have now proved active. Quaternary phenanthridine salts with free amino-groups are not only highly bactericidal *in vitro* but exert a curative action in trypanosome infections, especially those due to *T. congolense* and *T. vivax* (Browning, Browning, and Robb, 1940). The trypanocidal activity is greatest in 9-phenylphenanthridinium salts containing two amino-groups and appears to reach a maximum in 2 : 7-diamino-9-phenyl-10-methylphenanthridinium bromide (I ; $R'=R''=NH_2$, $R=H$; " phenanthridinium 1553," " dimidium bromide ") originally prepared by Walls (1947). High trypanocidal activity is also present when one of the amino-groups is situated in the 9-phenyl substituent (I ; $R = R' = NH_2$, $R'' = H$, " phenanthridinium 897," " phenidium chloride "). The γ-amino-group is particularly associated with trypanocidal action for the corresponding 3-amino-compounds are less effective (Walls *et al.*, 1945). In view of the activity of dimidium bromide and phenidium chloride, Caldwell and Walls (1948) prepared the third member of the triad (I ; $R = R'' = NH_2$, $R' = H$). In this compound both amino-groups are capable of ionic resonance with the heterocyclic *N*-atom, whereas in both the other compounds only one amino-group is suitably placed. Kumler and Daniels (1943) have suggested that ionic resonance of this type may be associated with parasiticidal properties. Similar compounds containing 6- or 8-amino-groups (II ; $R = R' = NH_2$) likewise possess two amino-groups capable of ionic resonance. The preparation of these phenanthridinium compounds with 2-, 6-, or 8-amino-groups has been accomplished by Walls (1947) by cyclising *o*-acylamidodiphenyls containing urethane substituents.



(I)



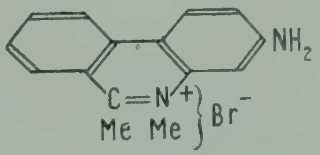
(II)



(III)

In a discussion of the relationship between trypanocidal action and structure in this series, Walls *et al.* (1946, 1947) point out that a high degree of activity is retained when the amino group R in (I) is replaced by a nitro-group. In view of the effect of the urethane group on therapeutic properties (Browning, Calver, Leckie and Walls, 1946) types were prepared by Caldwell and Walls (1948) in which R = NH . CO_2Et. Thus 7-amino-9-*p*-carbethoxyaminophenyl - 10 - methylphenanthridinium chloride (I ; R = NH . CO_2Et, R′ = NH_2) was prepared as well as compounds (I ; R = NH . CO_2Et, R″ = NH_2, R′ = H) and (III ; R = NH . CO_2Et, R′ = NH_2).

These quaternary salts have a high bactericidal power *in vitro*, an effect not reduced by the presence of blood. Some protect mice *in vivo* against *Streptococcus pyogenes*, the most effective being



Against *T. congolense* many salts have a well-marked action : (I ; R = R″ = NH_2, R′ = H) and (II ; R = R′ = NH_2[6-isomes]) but no convincing evidence is forthcoming of correlation between ionic resonance and trypanocidal action.

The phenanthridinium compounds most active against *T. congolense* possess two NH_2 groups, one of which may be situated in the benzene nucleus, as in No. 897.

Compounds with only one NH_2 group are more toxic and have less trypanocidal action. Acetylation of the NH_2 groups reduces both toxicity and therapeutic action. Nos. 897 and 1553, and the chloride corresponding to the latter, which is almost equally effective, differ in the position of one of the NH_2 groups. Neither drug, it may be noted, acts on *T. brucei* except in massive doses. Another compound of the same series, 3-carbethoxyamino-9(*p*-carbethoxyaminophenyl)-10 methyl phenanthridinium chloride (No. 1544) has no action on either *T. congolense* or *T. brucei* except in the highest doses, but nevertheless acts on *T. cruzi* in mice in a narrow dosage range (Browning, Calver, Leckie and Walls, 1946).

The activity of 7-amino-9(*p*-aminophenyl)-10 methyl phenanthridinium against *T. congolense* infections in mice was clearly demonstrated by Browning, Browning and Robb (1940) when given subcutaneously. This result was confirmed by Fulton and Yorke (1943), who found that the index $\frac{\text{MTD}}{\text{MCD}}$. was 4 when the drug was given by the subcutaneous route : but only 1 when the drug was given intraperitoneally. Administration by the oral and intravenous routes was unsatisfactory because of the poor therapeutic indices obtained. Although the subcutaneous route is superior to the intraperitoneal as a means of administration, ulcers are produced at the site of inoculation. Both Fulton and Yorke (1943), and Wien (1946) considered 7-amino-9(*p*-aminophenyl)-10 methyl phenanthridinium equal to 4 : 4′-diamidino dimethyl stilbene when tested against *T. congolense*. These workers treated infected mice at an early stage when trypanosomes in the blood were rather scarce. This circumstance would tend to lower the relative effectiveness of the phenanthridinium drug. Browning, Calver and Adamson (1948) find that to obtain consistent results it is necessary to pass the infection to fresh animals as soon as the parasites become abundant in the blood, that is to say at " acme." The sensitiveness to chemotherapeutic agents is preserved unchanged, apparently indefinitely, by such an acme strain (Browning and Calver, 1943 ; Calver, 1945).

The action of No. 897 (7-amino-9(*p*-aminophenyl)-10-methyl phenanthridinium chloride) and dimidium bromide on strains of *T. congolense* in mice is shown in the table on p. 441.

Results of Treatment at "Acme" of Mice Infected with *Trypanosoma congolense* (Browning *et al.*, 1948)

Drug.	Maximum tolerated dose in mgm. per 20 gm. of body wt.	Doses as a fraction of the maximum tolerated dose.	Number cured./Number treated in the case of strain		
			I	II	III
7-Amino-9(*p*-aminophenyl)-10-methyl phenanthridinium chloride (No. 897).	1	1 : 5–1 : 10	—	15/22	23/38
		1 : 15	—	34/75	0/4
		1 : 100	23/41	—	—
2 : 7 Diamino-9-phenyl-10-methyl phenanthridinium bromide (No. 1553 "Dimidium bromide").	1	1 : 40–1 : 50	—	—	23/37
		1 : 60–1 : 90	—	—	0/7
		1 : 100	—	25/28	—
		1 : 200	—	7/17	—
		1 : 400	14/24	—	—
4 : 4′-Diamidino-dimethyl stilbene	2	1 : 5	—	10/12	—
		1 : 8	—	—	20/25
		1 : 10	22/27	5/26	—
		1 : 12	—	—	0/17
		1 : 20	8/28	—	—
		1 : 30	2/24	—	—

Considerable variation in sensitiveness was seen in three different strains. With the phenanthridinium drugs the ratio of effectiveness for strains I, II and III was approximately 9 : 3 : 1. On the other hand, 4 : 4′-diamidino dimethyl stilbene yielded ratios which did not exceed 2 : 1.

The general conclusions to be drawn from laboratory investigations on phenanthridinium compounds with mixed substituents are shown in the table on page 442 : earlier work is fully discussed by Calver (1945).

Whereas a phenyl group in the 9-position is not essential for trypanocidal action, analogues with a methyl group in that position are less active against *T. congolense*. When one primary amino-group is present in the 7-position and another in the 9-phenyl ring it is immaterial, so far as toxicity and chemotherapeutic action are concerned, whether the latter amino-group occupies the *o*-, *m*- or *p*-position. The corresponding diacetamido-derivatives are all of low trypanocidal power : a nitro- instead of an amino-group in the phenyl ring causes only slight reduction in chemotherapeutic action. Carbethoxyamino-, and, still more markedly, acetamido-groups replacing the primary amino-group in the phenanthridine ring reduce toxicity and therapeutic activity.

Wien (1946), in studying nine phenanthridinium compounds in

TRYPANOCIDAL ACTION OF THE PHENANTHRIDINE SERIES (TYPE I)
(Walls *et al.*, 1947)

Compound.	Formula III		Therapeutic effect in mice : dose in mgm. per 20 gm. body weight.			
	Substituents.	Acid radical.	*T. congolense.*		*T. brucei.*	
			Dose.	Result.	Dose.	Result.
1600	7-NH . CO_2 . Et-9-Me	Br	3·3–2 1	+ + + + +	3·3	+
1596	7-NH_2-9-Me	Cl	1–0·5 0·2	+ + + + + + +	1	0
1605	2 : 7-NH_2-9-Me	Cl	2–0·2 0·1 0·05	+ + + + + + + + + + +	1	+
1598	2 : 7-NH . CO_2Et-9-Me	Cl	1·66 0·83–0·55	+ + + + + + +	1·66	+
1581	2 : 7-NH . COMe-9-Me	Cl.	4	0	4	0
1593	7-NH . COMe-9-p-C_6H_4 . NH_2	$MeSO_4$	4 2	+ + + + +	4	0
1595	7-NH . COMe-9-p-C_6H_4 . NH . CO_2Et	Cl	4 2	+ + + + +	4	0
1592	7-NH . CO_2Et-9-p-C_6H_4 . NH_2	Cl	2–0·2 0·1	+ + + +	2	0
1594	7-NH . CO_2Et-9-p-C_6H_4 . NHCOMe	Cl	4	+ + +	4	0
1586	7-NH_2-9-p-C_6H_4 . NO_2	Cl	2–0·033 0·017	+ + + + + +	2	+
1590	7-NH . COMe-9-o-C_6H_4 . NH_2	Cl	0·5	0	0·5	0
1579	7-NH . CO_2Et-9-o-C_6H_4 . NH_2	Cl	2–0·1 0·066 0·033	+ + + + + + + 0	2	0
1591	7-NH . CO_2 . Et-9-o-C_6H_4 . NH . COMe	Cl	5 1·66	+ + + + 0	5	0
1587	7-NH_2-9-o-C_6H_4 . NO_2	Cl	1·43–0·66 0·033 0·017	+ + + + + + +	1·43	0
1588	7-NH_2-9-o-C_6H_4 . NH_2	Cl	0·2–0·02 0·01 0·007	+ + + + + + + +	2	0
1589	7-NH . COMe-9-o-C_6H_4 . NH . COMe	Cl	3·3	0	3·3	0

+ + + + = Sterilisation of infection.
+ + + = Cure in a proportion of mice.
+ + = Absence of parasites in the blood for at least ten days.
+ = Absence for a few days.
0 = No effect.

mice, came to the conclusion that the replacement of amino by amidine groups led to a loss of activity : the substitution of the 9-(4′-aminophenyl) by the 9-(3′-pyridyl) grouping also leads to a reduction in activity. Similarly, the introduction of an unsaturated linkage (p-dimethylaminostyryl) between phenyl at C 9 and the phenanthridine nucleus causes a reduction in activity.

T. congolense in mice is highly susceptible to phenanthridine compounds, *T. brucei* is much less affected : on the other hand, a compound having a weak action on *T. congolense* may similarly have a weak action on *T. brucei* whereas compounds more active *T. congolense* show no action on *T. brucei.*

Pharmacologically the phenanthridinium compounds have received comparatively little study. Wien (1946), however, has found that dimidium bromide has a depressant action on the circulation and respiratory rate. In large doses in mice it has a toxic action on the kidneys and liver.

Phenanthridinium Compounds in Cattle Infected with *T. congolense.* Two phenanthridinium compounds have now been tested in cattle infected with *T. congolense.* S 897, or phenidinum, is given intramuscularly or intravenously at the rate of 2 mgm. per kgm. of body weight: it is soluble only to the extent of 1 per cent. in water. Hornby *et al.* (1943) prefer to give a large intramuscular dose such as 15 ml. of a 0·5 per cent. aqueous solution per cwt. of body weight or an intravenous injection of 10 ml. of a 1 per cent. solution per cwt. It is noteworthy that among fifteen experimentally infected calves the only three to relapse were treated five weeks or less after infection. Although it is highly trypanocidal, some of the earlier samples of the drug tended to cause lameness, œdema of the scrotum and, if given intramuscularly, sloughing of the tissues (Carmichael and Bell, 1944). Dimidium bromide, S 1553, appears in many ways to be more effective (Stewart, 1946; Carmichael and Bell, 1944; Bell, 1945; Randall and Laws, 1947; Wilson, 1948; Guyaux, 1948). It is prepared as a deep-red, crystalline powder, freely soluble in water. Bell found that the minimum curative dose for cattle was 0·8 mgm. per kgm. of body weight, the chemotherapeutic index being 6·25. Relapses, if they occur, are generally seen in from sixteen to forty-three days, but occasionally they may be delayed as long as ninety-three days. Guyaux (1948), after giving 1 mgm. per kgm. intravenously or subcutaneously, had a relapse rate of 7·84 per cent. It appears that all phenanthridinium compounds are not as effective in early as in well-established cases and less effective against some strains than others (Hornby *et al.*, 1943; Randall and Beveridge, 1946; Randall and Laws, 1947). Delay till twenty-four days after infection is beneficial, but after thirty-four days the relapse rate is increased, at any rate with a dose rate of 1·0 mgm. per kgm. of body weight. Browning and Calver (1943) had already drawn attention to the relationship in mice between the stage of infection and the response to drugs. The anæmia due to *T. congolense* infections is then more difficult to overcome (T-W-Fiennes *et al.*, 1946).

The assumption that immunity reactions assist the action of phenanthridinium compounds is strengthened by the fact that for grade cattle the minimum curative dose is 1·25 mgm. per kgm. (Barnett, 1946), whereas for indigenous zebu cattle it is 0·8 mgm. to 1 mgm. per kgm. (Bell, 1945; Randall and Laws, 1947). Relapse strains may exhibit some resistance to phenidinum and dimidium bromide, but Guyaux (1948) finds that doses of 2 mgm. per kgm. of body weight are able to control relapse infections in the majority of instances. Hornby *et al.* (1943) report that strains resistant to phenidinum are not resistant to other trypanocidal compounds such as stibophen.

There is general agreement that the majority of infections due to *T. congolense* can be rapidly cured by dimidium bromide. Guyaux (1948), in a large herd of cattle, had a cure rate of 88·23 per cent. *T. congolense* infections in dogs have also been cured by a single injection of 4 ml. of a 2 per cent. solution (T-W-Fiennes, 1947). According to Guyaux (1948), the highly fatal infection of pigs caused by *T. simiæ* also responds to dimidium bromide in a dose of 1 mgm. per kgm. *T. vivax* in cattle also reacts, but *T. brucei* is unaffected.

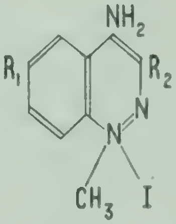
Toxic reactions have been noted following the injection of phenanthridinium compounds. With doses of 6 mgm. per kgm. of body weight and above there is salivation, shallow breathing, muscular inco-ordination and finally coma and death: doses of 4 mgm. per kgm. of body weight cause mild symptoms. Locally, after subcutaneous injection, there may be some swelling, loss of hair or even sloughing of the tissues. A more important finding is the occurrence of photosensitisation coming on some six weeks after injection of the drug and usually causing death in about a week. The distribution of photosensitisation in Africa is somewhat irregular, even in Uganda, where most of the cases have been reported. Guyaux (1948), in the Belgian Congo, was untroubled by it.

It is thus possible to summarise the field trials on African cattle with phenanthridinium compounds, especially dimidium bromide, by saying that cure often results from a single dose at the rate of 1·5 to 2 mgm. per kgm. given subcutaneously or intravenously. In general, this dose is remarkably well borne by cattle, although given subcutaneously it may cause some local damage, the severity of which is variously assessed by different workers. Randall and Beveridge (1947), for instance, state that the local reactions combined with photosensitisation are such as to frighten the African stock owner and to destroy his confidence in anti-trypanosomiasis veterinary procedures. Symptoms attributed to photosensitisation have been so irregularly distributed as to suggest contributory factors apart altogether from the drug; possibly some leguminous plant in association with the drug is responsible. Similar sensitisation phenomena have been recorded apart altogether from treatment, and nothing comparable has been seen in smaller laboratory animals (Brownlee *et al.*, 1947).

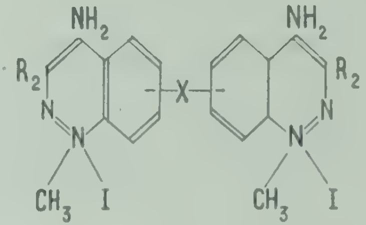
Apart from toxic reactions in cattle, the drawback to the use of phenanthridinium compounds is the development in the field of resistant strains, as recorded by Randall and Laws (1947). Trypanosomes rendered resistant both to phenidium and dimidium bromide maintain their resistance to drug treatment after subinoculation into cattle. Such phenanthridinium-resistant strains, however, are curable by means of stibophen. Curiously enough drug-fast strains in mice have not been produced by any of the means usually employed to cause drug fastness (Browning, 1949). The possibility of relapsed infections creating reservoirs of trypanosomes incurable by phenanthridium compounds renders a policy of mass treatment with these drugs somewhat hazardous. However, more than two million doses of dimidium bromide have already been used in Africa and very considerable outbreaks of cattle trypanosomiasis have been controlled.

Cinnoline Derivatives. Keneford *et al.* (1948) carried out investigations to determine whether trypanocidal activity is retained in simplifications of the phenanthridinium molecules. They have synthesised quaternary salts of a variety of mono- and diamino-cinnolines, quinazolines and quinolines. In contrast to the highly active phenanthridinium compounds, bicyclic heterocyclic quaternary salts had little or no trypanocidal action, possibly because the molecular weights of the cations of these bicyclic salts may be below the minimum necessary for activity.

Two of the compounds prepared were 4 : 6-diamino-1-methylcinnolinium iodide (I ; $R_1 = NH_2$; $R_2 = H$) and its 3-methyl analogue (I ; $R_1 = NH_2$; $R_2 = Me$). These diamines were inactive in the pure state but the crude reduction products of the corresponding 6-nitro-salts (I ; $R_1 = NO_2$; $R_2 = H$ and Me) were active, the trypanocidal power varying according to the conditions used for reduction. The most favourable result was a chemotherapeutic index of about two against *T. congolense* in mice, the maximum tolerated dose being about 4·5 mgm. intraperitoneally and the minimum curative dose about 2·25 mgm. per 20 gm. body weight, with a crude reduction product of I ($R_1 = NO_2$; $R_2 = Me$). Tested by the same technique, the best-known phenanthridinium compounds, dimidium bromide (1553) and phenidium chloride (897) have indices of fifteen and three respectively.



(I)



(II)

It is suggested that trypanocidal activity may be due to the incidence of side-reactions accompanying the conversion of the nitro- to the amino-quaternary salts ; and assuming that the same side-reaction is responsible for the development of activity in each of the two cases, the active components may possibly be the azo-compounds (II ; $X = 6 : 6' - N = N$). Compounds of type (II) possess one of the striking features of many non-arsenical trypanocidal compounds in that they are symmetrical molecules containing two identically constructed cyclic units.

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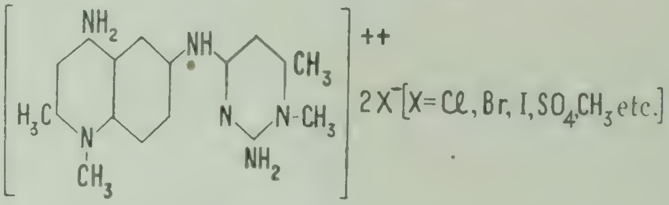
Antrycide

A new compound, especially active against cattle trypanosomes, has been described by Curd and Davey (1949): it is 4-amino-6-(2′-amino-6′-methyl pyrimidyl-4′-amino) quinaldine-1 : 1′-diametho (chloride, bromide, iodide).



Antrycide salts

The trypanocidal effects of antrycide chloride when given subcutaneously are shown in the table on page 449.

Against an infection in mice due to *Trypanosoma congolense*, a single dose of 1 mgm. of antrycide chloride per kgm. of body weight was curative. Half this dose effected a proportion of cures with the Busimbi strain, but with one strain from Kenya a dose of about 2·5 mgm. per kgm. was required to produce consistent cures in mice.

Antrycide salts also possess considerable action against trypanosome infections in the larger animals. A single subcutaneous injection cured infections due to *T. congolense*, *T. vivax* and *T. brucei* in cattle, *T. brucei* in horses, donkeys and dogs, and *T.*

TRYPANOCIDAL ACTION OF ANTRYCIDE CHLORIDE AGAINST VARIOUS SPECIES OF TRYPANOSOMES

Dose (mgm./kgm.).	*T. rhodesiense* (Tinde).	*T. brucei* (Liverpool).	*T. congolense* (Busimbi).	*T. evansi* (India).	*T. evansi* (Sudan).	*T. equiperdum*.	*T. equinum*.
25	C	—	—	—	—	—	—
12·5	C	—	—	—	—	—	—
5	C	C & R	—	C	C & R	—	C
2·5	—	R	—	—	—	—	—
1·25	—	—	C	C	C & R	C	C
1·0	—	—	C	—	—	—	—
0·5	—	—	C & R	C & R	R	C	C & R
0·25	—	—	R	—	—	C & R	—
0·125	—	—	R & DD	—	—	—	—

C = cure ; blood free for 28 days ; R = relapse ; DD = delayed death.

evansi in camels. Wilson (1949a, c) found that antrycide was able to act also on *T. simiæ* in pigs : it was curative in doses of from 3 to 5 mgm. per kgm. It exerts a prophylactic action for some months, but the limit of activity, which may be as long as six months, has not yet been determined accurately. However, antrycide apparently induces drug-resistance both in *T. simiæ* and, of greater importance, in *T. vivax* and *T. congolense* (Wilson, 1949a, b). With therapeutic doses the toxicity of antrycide is low. It may cause a slight swelling at the point of injection when given subcutaneously, and a temporary reduction in the milk yield has been reported (Davey, 1949). It has no action on carcass meat when given prophylactically. Some fatal cases of poisoning have been reported in cattle by Wilson (1949b). A dose of 15 mgm. per kgm. of antrycide sulphate causes a death rate of 5 per cent., and a dose of 30 mgm. per kgm. is invariably fatal. A dose of 11 mgm. per kgm., only twice the full curative dose, may occasionally be followed by symptoms of poisoning and death. The symptoms of toxicity, twitching or trembling of the labial and nasal muscles, a chewing movement of the lower jaw with increased salivation and grinding of the teeth, were very characteristic and developed ten to thirty minutes after administration of the drug, At the same time the animal became restless, with incoordination and increased skin sensitivity. Occasionally the whole head appeared to tremble and the triceps of the shoulders and biceps on the flanks showed intermittent twitching. The heart and respiratory rate were increased and collapse occurred with death in a few hours. Some animals recovered, but about forty-eight hours later showed profuse bloody diarrhœa and loss of condition with death in from six to twenty days later. At necropsy, when death occurred soon after the dosage, there was marked inflammation of the abomasum, small intestines and rectum, with enlargement of the gall bladder, hæmorrhagic inflammation of the pancreas and acute inflammation of the kidneys. In more chronic cases the abomasum was ulcerated while the mucous membrane of the duodenum and cæcum was greatly thickened.

In mice the following results were obtained after injection of antrycide sulphate.

RESULTS OF INJECTION OF ANTRYCIDE SULPHATE IN MICE
(Wilson, 1949b)

Dosage, mgm. per kgm. . .	10	15	20	25	30
No. of mice used .	18	12	22	14	10
No. died . .	0	2	9	5	10

In those mice which died, death was preceded by a short period of excitement : the mice moved uneasily round the cage, gave a few leaps into the air and died. Those mice which recovered showed a brief period of restlessness and accelerated breathing, but were normal thirty to sixty minutes after injection.

The following method of estimating antrycide has been described by Spinks (1949).

Special Reagents. (1) Stock solution of eosin (500 mgm.) in saturated sodium bicarbonate (A.R., 500 ml.). This stock solution is purified by shaking it four times with solvent mixture. (2) Buffered eosin reagent. The stock solution is diluted 1/25 with saturated sodium bicarbonate solution. (3) Solvent mixture. Dilute 200 ml. of re-distilled butanol to 1,000 ml. with B.P. chloroform. (4) Antrycide stock standard solution, 100 mgm./100 ml. (referred to ion). Dissolve 134·5 mgm. of dichloride dihydrate in distilled water and dilute to 100 ml. (5) Antrycide working standard, 0·05 mgm./100 ml. Dilute the stock standard when required.

Special Apparatus. Coleman electronic photofluorimeter, model 12A.

Procedure. Dilute 1·2 ml. of plasma to 10 ml. with distilled water. Add 2 ml. of 15 per cent. trichloroacetic acid, mix well and allow to stand at least ten minutes. Centrifuge, and transfer 10 ml. of the clear upper layer to a 60-ml. glass-stoppered bottle. Add 1 ml. of *N*-sodium hydroxide, 2 ml. of buffered eosin reagent, and 12 ml. of solvent mixture. Shake vigorously for three minutes, and allow to stand until the lower layer has separated. Remove the latter, and clarify it by filtering through Whatman No. 1 or similar semi-fine paper into a Coleman fluorimeter cuvette. Read the fluorescence against a blank and standards in the Coleman fluorimeter. These are prepared by adding 0, 0·1, 0·3, 0·6, 1·0, 2·0 and 3·0 ml. of the Antrycide working standard to 30-ml. bottles, treating with 2 ml. of buffered eosin reagent and 12 ml. of solvent mixture, and continuing the estimation as described above. Coleman PC 9 (pale yellow) visual filter and Coleman B 1 (low sensitivity) or B1S (high sensitivity) ultra-violet filter are used in the fluorimeter.

The procedure measures down to 50 μgm. of Antrycide/litre of plasma with satisfactory accuracy (± 10 per cent.) and down to 20 μgm./litre with fair accuracy (± 25 per cent.). Under carefully controlled conditions, concentrations from 5 to 20 μgm./l. can be detected as "traces." Antrycide added to normal plasma is not completely recovered. The loss of about 20–25 per cent. is due to precipitation of Antrycide with protein, and is increased if larger volumes of plasma are used. The method has a considerable degree of specificity, since tertiary bases appear not to react under the conditions described, and many quaternary bases (for example, *d*-tubocurarine chloride, Dimidium bromide and 4 : 6-diaminoquinaldinemethochloride) give only weakly fluorescing extracts. However, it is not yet possible to state that the method does not measure metabolites of the drug.

Although antrycide gives a blue fluorescence in ultra-violet light this property is not sufficiently sensitive to measure the low concentrations usually found in plasma. Antrycide has no action on trypanosomes in the salivary glands of tsetses.

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Other Trypanocidal Compounds

(1) **Antibiotics.** The antibiotics so far investigated have shown little action on pathogenic protozoa. Waksman *et al.* (1941) and Robinson (1943) believed that streptothricin and actinomycin prolonged the lives of mice infected with *Trypanosoma equiperdum*. Schatz *et al.* (1946) found that by the method of soil enrichment (Waksman and Schatz, 1946) they were able to obtain a fungus which was trypanocidal *in vitro* to *T. equiperdum*. Frozen and dried trypanosomes were added to soil eleven times in three months and at the end of this period the filtrate of a *Phycomyces*, belonging either to the genus *Phytophera* or *Pythium*, was found to inhibit *T. equiperdum*. The active factor is produced in a glucose peptone broth as well as on a synthetic glucose-asparagin medium : it is then adsorbed on norit and eluted with organic soluents. It is soluble in alcohol and water. Higher concentrations are obtained by treating old cultures with pyridine or benzene. The extracts varied in activity but usually immobilised trypanosomes in dilutions of 1 in 100 to 1 in 1,000. There is some evidence that the *Phycomyces* factor is a lipid. Unfortunately no action is found *in vivo* against *T. equiperdum* though the toxicity is no greater than that of alcohol. Clavacin inactivitates *T. equiperdum* in a dilution of 1 in 16,600, fumigacin 1 in 500, and streptomycin 1 in 200.

Weinman (1943) found that tyrocidine inhibited the growth of *T. cruzi* and *T. lewisi in vitro* in a concentration of 5·0 gm. per ml. but gramicidin was inactive in a concentration of 1·0 gm. per ml. The majority of workers have found penicillin inactive (Neghme, 1945). Pizzi (1945) used penicillin as an aid to obtaining bacteria-free cultures of *T. cruzi*, and Talice (1945) failed to influence the course of infection in animals and in one human patient by penicillin. Adler *et al.* (1948), however, found that crude penicillin inhibits the growth of *T. cruzi*, Torrealba (1945) and Earle (1946) also believe that penicillin may be of value in Chagas' disease. Earle found that 50,000 units cleared the blood in one case, the patient remaining in good health for thirteen months. Nelson (1945) reported that penicillin was without action on *T. rhodesiense*. Anderson *et al.* (1946) found that subtilin, an antibiotic obtained from *Bacillus subtilis* by Jansen and Hirschmann (1944), caused immediate lysis of *T. equiperdum* in a dilution of 1 in 2,000. Survival of infected mice, however, was not prolonged when 80 to 160 mgm. per kgm. of subtilin was given intraperitoneally.

According to Tedeschi (1948), coriophiline, an antibiotic from *Penicillium coriophilum*, is active in immobilising and lysing trypanosomes: similar effects are obtained with pigments from *Aspergillus niger*, which are active both against *Staphylococcus aureus* as well as *T. lewisi* and *T. gambiense*. The trypanosomes are rendered motionless and after a time become deformed and aggregated: red cells are hæmolysed. Doses of 3 mgm. per 100 gm. of body weight are, however, active and non-toxic in mice. Streptomycin is useless against trypanosomes *in vivo* (Merchant, 1947). Adler and Bichowsky (1946) brought forward evidence that biotin concentrates contain an antibiotic not biotin, which inhibits the growth of *T. lewisi* and *T. cruzi*. Caldwell and György (1947) believe that biotin deficiency causes an intense and prolonged infection in rats suffering from *T. lewisi*: there is a diminished production of ablastin, complement, and trypanolysin.

(2) **Nitrobenzoic Acid Compounds.** The trypanocidal action of 3-hydroxybenzoic acid was originally demonstrated in 1912 by Morgenroth and Rosenthal, but it was not till 1939 that Mayer and Oechslin, and Rosenthal, Bauer and Elvove showed that 4-nitrobenzoic acid had a slight action against *T. equiperdum*. Rosenthal and Bauer (1941) also studied the possible activity of 3-nitrobenzoic acid which is more active *in vivo* against *T. equiperdum*, while *in vitro* concentrations up to 0·1 per cent. cause

considerable swelling of the trypanosomes with decrease in motility after one hour at room temperature. The *ortho* isomer is without action, but a number of derivatives of the *meta* derivative have some action *in vivo* though none is as active as the sodium salt of 3-nitrobenzoic acid.

THE ACTION OF 3-NITROBENZOIC ACID AND ITS DERIVATIVES ON *T. equiperdum* IN MICE (Rosenthal and Bauer, 1941)

Compound.	Route of administration.	Gm./kgm. × days.	Mean survival time in days.
Sod. 3-nitrobenzoate	Subcutaneous	0·5 × 6	13·3
	Oral	0·5 × 6	5·1
3-Nitrobenzoic acid (in oil)	Subcutaneous	0·5 × 4	6·8
3-Nitrobenzyl alcohol	,,	0·5 × 6	5·8
	,,	0·5 × 2	3·9
3-Nitrobenzyl chloride	Oral	1·0 × 5	5·7
3-Nitrotoluene (in oil)	Subcutaneous	0·25 × 3	3·6
3-Nitrobenzoate methyl	,,	0·5 × 4	8·2
3-Nitrobenzoate ethyl	,,	0·5 × 4	6·2
3-Nitrobenzaldehyde	,,	0·5 × 3	3·5
	,,	0·5 × 3	3·0
Sod. 3-hydroxybenzoate	Oral	1·5 × 4	5·5
3-Bromobenzoate	Subcutaneous	0·25 × 3	5·1
Sod. 4-nitrobenzoate	,,	0·5 × 6	4·9
Nicotinic acid	,,	1·0 × 3	4·0
Controls		—	3·1

The nitro-group is apparently not essential for trypanocidal action for some activity is shown by 3-hydroxy- and 3-bromobenzoic acid as well as by nicotinic acid. The importance of the carboxy group is proved by the fact that only those derivatives are active which can be converted into it. Replacement by OH or substituted OH groups, or by Cl, SO_3H or SO_2NH_2, abolishes trypanocidal action.

(3) **Antibiotic Lactones and their Analogues.** *Furacin*, 5-nitro-2-furaldehyde semicarbazone, is active against a number of Gram-positive and Gram-negative bacteria; it has been found by Dodd (1946) to be active in infections due to *T. equiperdum* in

rats and mice. The LD 50/CD 50 in mice is 9 while in rats the LD 100/CD 100 is 8. A curious phenomenon seen in the treatment of bacterial infections is that while doses of about 150 mgm. per kgm. produce maximum effects, at higher doses the results are significantly poorer: in trypanosome infections doses above 100 mgm. per kgm. for rats and 150 mgm. per kgm. for mice have not been tried. With maximal doses there is severe hepatitis and extensive degeneration of the tubular epithelium of the kidney.

Acute and chronic toxicity tests in rats, mice, and monkeys showed that furacin has a very low toxicity. Several persons took 100 mgm. orally three times a day without symptoms. No changes were seen in the blood or urine. When the dose was increased to 2 to 4 gm. daily some 20 per cent. of people suffered from nausea (Krantz and Evans, 1945).

Rubin (1948) and Giarman *et al.* (1949) confirm the action of furacin on *T. equiperdum* in doses of 500 mgm. per kgm. of mice given either subcutaneously or *per os*. Furfuryl alcohol and furfural were active, but less so. Furoic acid is ten times as potent as furfural *in vitro* but quite inactive *in vivo*: this may, however, be due to its greater toxicity. In the lactone series as a whole, and Rubin has tested a total of forty-four compounds, the active members are characterised by a Y or S ring with a carbonyl group capable of acting as a hydrogen donator. Unsaturation in either type of ring increases activity. With the S lactones a multiple, conjugated system of double bonds increases activity still more. Substituent groups which do not themselves give some action, such as penicillic acid, almost invariably decrease activity, in accordance with the observation that interfacial penetrating power is depressed by side-chains.

Three lactones were active *in vivo*: 2-pentene-1, 4-olid, penicillic acid, and coumalinic acids: *in vivo* and *in vitro* activities are not correlated. Hydrogenated furans are inactive and nitrogen analogues are inactive and toxic. Trypanocidal action is not quantitatively related either to toxicity or to antibacterial action. Hexenolactone was found by Hauschka *et al.* (1945) to inhibit the growth of *T. cruzi in vitro*: unfortunately *in vivo* it is without action. Rubin (1947) believes that though some natural and synthetic lactones are trypanocidal, the level of the activity depends both on the nature of the substituents and the position of the unsaturated linkages. The *in vitro* trypanocidal activity of " parasorbic " acid can be inhibited by β-alanine and to some extent by α-alanine : calcium pantothenate and pantolactone have no such action.

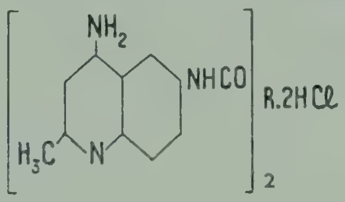
(4) **Nitrogen Mustard.** Nitrogen mustard, methyl-bis (β-chloroethyl) amine has been found by Chen (1948) to have, besides its general toxic action, a specific effect on the reproduction of trypanosomes. Low concentrations, below 10^{-3} molar, produce a mitotic inhibition without affecting the glucose metabolism of the parasites. Cysteine antagonises the effect of nitrogen mustard.

(5) **Prodigiosin.** Prodigiosin, the red dye of *Serratia marcescens*, was found by Fischl (1935) to have some action against trypanosomes *in vivo.* Rubin (1947) finds prodigiosin active only *in vitro* against *T. equiperdum.*

(6) **Quinaldine Derivatives.** A series of twenty-three quinaldine derivatives was examined by Goble (1948) for trypanocidal action *in vivo.* These quinaldines are characterised by the presence of two identical 2-methyl-4-aminoquinoline constituents connected through the 6-position by bridges derived from urea, straight and branched-chain dicarboxylic acids or polymethylene glycols. They are close relatives of the quinaldine derivative Bayer 7602Ac (Mazza *et al.,* 1937).

Compounds in which the bridge between the quinoline groups is not a branched chain (the carbamide " surfen," the malonamide through the sebacamide, and the quinolyloxy alkanes) are active against the *brucei* group (*T. brucei*, *T. gambiense*, *T. rhodesiense*) but inactive against *T. cruzi.* The branched chain compounds, disubstituted malonamides, in which the branches are short alkyl chains (methyl-methyl groups through ethyl-propyl and ethyl-allyl groups) are only weakly active against the *T. brucei* group but their activity against *T. cruzi* increases directly with the length of the alkyl chains. The peak of activity against *T. cruzi* is reached in those drugs in which one branch is either propyl or allyl and the other branch contains three or more carbons in either aliphatic or aromatic arrangements. These latter compounds are completely inactive against the *T. brucei* group. None of the amides or ethers studied had any appreciable activity in infections due to *T. congolense.* But a close relative, the cyanuric acid derivative, *bis*-(2-methyl-4-amino-6-quinolyl)melamine, variously known as Surfen C or Congasin, is active against *T. brucei* and *T. congolense.*

TRYPANOCIDAL ACTIVITY OF 4,6-DIAMINOQUINALDINE
(Pratt and Archer, 1948)



R.	Formula.	Activity against	
		T. cruzi.	*T. brucei.*
CH_2	$C_{22}H_{22}N_6O_2 . 2HCl . 5H_2O$	—	+
$(CH_2)_3$	$C_{25}H_{26}N_6O_2 . 2HCl . 4H_2O$	—	+
$(CH_2)_4$	$C_{26}H_{28}N_6O_2 . 2HCl$	—	+ + + +
$(CH_2)_5$	$C_{27}H_{30}N_6O_2 . 2H_2O$	—	+
$(CH_2)_7$	$C_{29}H_{34}N_6O_2 . 2HCl . H_2O$	—	+
$(CH_2)_8$	$C_{30}H_{36}N_6O_2 . 2HCl$	—	+
$(CH_3)_2C$	$C_{25}H_{26}N_8O_5 . 2HCl . 3H_2O$	+	+
$(CH_3)(C_2H_5)C$	$C_{26}H_{28}N_6O_2 . 2HCl . 2H_2O$	±	+
$(CH_3)(C_3H_7)C$	$C_{27}H_{30}N_6O_2 . 2HCl . 3H_2O$		
$(C_2H_5)_2C$	$C_{27}H_{30}N_6O_2 . 2HCl . H_2O$	+ + +	+
$(C_2H_5)(C_3H_7)C$	$C_{28}H_{32}N_6O_2 . 2HCl . 3H_2O$	+ + +	+
$(C_2H_5)(C_3H_5)C$	$C_{28}H_{30}N_6O_2 . 2HCl . 5H_2O$	+ + +	+
$(C_3H_7)_2C$	$C_{29}H_{34}N_6O_2 . 2HCl$	+ + +	+
$(C_3H_7)(C_3H_5)C$	$C_{29}H_{32}N_6O_2 . 2HCl . H_2O$	+ + +	—
$(C_3H_5)_2C$	$C_{28}H_{30}N_6O_2 . 2HCl$	+ + + +	—
$(C_4H_9)_2C$	$C_{31}H_{38}N_6O_2 . 2HCl . 5H_2O$	—	—
$(C_3H_5)(C_4H_3SCH_2)C$	$C_{31}H_{30}N_6O_2S$	+ + +	—
$(C_3H_5)(C_6H_5CH_2)C$	$C_{33}H_{32}N_6O_2 . 2HCl . 2H_2O$	+ +	—

(7) **Diaminoquinaldines.** The trypanocidal activity of a number of 4,6-diaminoquinaldines has been studied by Pratt and Archer (1948). Their activity in mice against *T. cruzi* and *T. brucei* is shown in the table. As a group they were inactive against *T. congolense.* Only the adipamide showed more than weak activity against *T. hippicum* and *T. equiperdum.* Against *T. brucei* almost all the straight chain amides exhibited some activity, the adipamide

($C_{26}H_{28}N_6O_2 . 2HCl$) being strongly active whereas the disubstituted malonamides were slightly or completely ineffective. On the other hand, against *T. cruzi* the malonamides exhibited pronounced activity, but the straight amides were ineffective.

(8) **8-Aminoquinolines.** Goble (1949) found that 8-aminoquinolines have some activity against *T. cruzi* in mice. Of the compounds examined pentaquine, 8-(*iso*propylaminoamylamino)-6-methoxyquinoline, was the most effective in doses of 15 and 30 mgm. per kgm. of body weight: 8-(diamylaminoethylamino)-6-methoxyquinoline triphosphate was inert at 60 and 30 mgm. per kgm. of body weight. The following results were obtained by intraperitoneal inoculation.

Compound.	Dose level (mgm. per kgm.).	Mean survival time (days).	Percentage surviving.	
			Day last control died.	60 days.
Untreated controls . . .	—	10·7	all dead in 13 days.	
8-(diethylaminoethylthioethylamino)-6-methoxyquinoline monocitrate . .	40	10·3	10	0
8-(diethylaminoethylthiopropylamino)-6-methoxyquinoline monocitrate . .	40	11·1	0	0
8-(diethylaminopropylthiopropylamino)-6-methoxyquinoline monocitrate . .	60	13·5	40	0
8-(dibutylaminopropylamino)-6-methoxyquinoline triphosphate	60	10·5	20	0
8-(nicotinylamino)-6-methoxyquinoline (base) .	80	11·5	10	0
8-(dimethylamino*iso*butylamino)-6-hydroxyquinoline monosulphate (base) . .	100	10·6	20	0
Pamaquin . . .	15	13·2	40	0
	20	15·6	60	0
Pentaquine 8-(*iso*propylaminoamylamino)-6-methoxyquinoline monophosphate .	15	14·7	60	0
	30	25·3	100	10

None of the 8-aminoquinolines tested has any action on *T. congolense* nor among those tested on *T. brucei*. Nevertheless the demonstration of trypanocidal activity against *T. cruzi* among the 8-aminoquinolines is of considerable interest. It demonstrates that this group has some action both on malarial plasmodia and trypanosomes: it reveals a new chemical group in which to seek compounds active against *T. cruzi* and it introduces an agent for the treatment of Chagas' disease which is active on oral administration.

(9) It has long been recognised that normal human serum contains trypanocidal substances. Some light is possibly thrown on this finding by the discovery that the respiration of *T. equiperdum* is inhibited at least partially by basic polypeptides (Reiner *et al.*, 1942). Further investigations by Bloom and Blake (1948) show that tissue polypeptides combine with trypanosomes which are then incapable of metabolising ribonucleic acid.

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THE CHEMOPROPHYLAXIS OF TRYPANOSOMIASIS

In view of the increasing percentage of cases of sleeping sickness which are found to be naturally arsenic-resistant, attempts have been made to protect against infection by the prophylactic injection of drugs which have a trypanocidal action. As sleeping sickness, whenever possible, should be diagnosed before the central nervous system is involved it is highly important that the infection should be actually prevented and not merely suppressed.

Prophylaxis with Suramin

In animals treated prophylactically with suramin the disease often runs an atypical course, trypanosomes being absent from the peripheral blood (Corson, 1934; van Hoof *et al.*, 1940). In experiments with human volunteers Duke (1936) found three, possibly five, instances of cryptic infection. Fulton (1944) observed a somewhat similar condition when mice were not completely protected by aromatic diamidines.

In order to be of value as a chemoprophylactic in man it is essential that a trypanocidal titre should be maintained in the blood stream for a considerable period. Tervalent arsenicals will produce a high trypanocidal titre for a short period only and are thus valueless. Quinquevalent arsenicals cause a trypanocidal titre for a longer period and, against nagana, tryparsamide was shown by Levaditi *et al.* (1926) to exert a prophylactic action when given by mouth to rabbits. In the field, Fourche and Haveaux (1931) gave 2 gm. of tryparsamide intravenously and found that protection persisted for as long as six months, the number of fresh infections being twelve times less than among untreated controls. In view, however, of the toxicity of tryparsamide and, of even greater importance, the high percentage of patients in Africa now met with who are infected by arsenic-resistant strains, the use of arsenicals as a prophylactic is of course out of the question.

The possibility of chemoprophylaxis by suramin was investigated by Kleine and Fischer (1922) in view of the long period during which high concentrations of suramin survive in the blood. The injection of 0·15 gm. to monkeys protected up to two months against the bite of infected flies. In mice, Launoy *et al.* (1929a and b) found that the length of protection by suramin varies directly with the amount of the drug injected. The same relation holds good for the cat. In rats, Corson (1934) reported that a dose of 0·015 gm. per kgm. of body weight failed to protect against a fly-transmitted strain of *T. rhodesiense* even for twenty-one days while 0·03 gm. per kgm. was not effective for forty days. Kolmer

(1931) attempted to protect against infection by oral administration of suramin and of arsenicals.

Duke (1934) gave injections of from 0·158 to 0·023 gm. per kgm. of body weight to three monkeys : these animals could be reinfected with *T. rhodesiense* sixty-seven, sixty-nine and seventy-four days later.

In domestic animals Pfeiler (1922) used 6 gm. of suramin during the covering season in two doses of 3 gm., at an interval of eight days. Tejera (1924) found 2 gm. repeated every six months of value in horses in Venezuela. Results obtained by Kleine (1924) and Berg (1925) in cattle were disappointing, for even 10 gm. for a bullock of 5 cwt. failed in some instances to prevent infection.

Against surra, Baermann (1922) obtained protection for thirty to forty days by injecting 2 gm. to 6 gm. of suramin. Edwards (1926) also obtained complete protection against surra for twenty-two days by an intravenous injection of 7·5 gm. per 1,000 lb. of body weight.

In man, Duke (1934) showed that a single dose of 1·0 gm. of suramin given intravenously will protect against infection with *T. rhodesiense* transmitted by the bites of tsetse flies ; a second dose three weeks later enhanced the protective action. The protection afforded appears to vary somewhat with different strains of *T. rhodesiense*, possibly because of the differing power which these strains possess of propagating themselves in man. The protection against *T. rhodesiense* is greater than against *T. gambiense*. An injection of 1·0 to 1·5 gm. should probably be given every three months, a conclusion in agreement with that reached by Mayer (1928) that Europeans should receive intravenous injections of 2 gm. every three months and Africans injections of 1 gm.

The prophylactic use of suramin and also of orsanine was at first studied in man chiefly by Belgian and French investigators, though during the war of 1939–45 injections of suramin were given to more than 500 troops serving in a highly infected area in the Gambia. There were no cases of infection among those prophylactically inoculated with intramuscular injections of 1 gm. In the Anglo-Egyptian Sudan those wishing to visit certain endemic areas in Uganda and the Belgian Congo must submit to a prophylactic inoculation with suramin. A further injection is given when the visitor returns. Suramin has been given also to some 800 fly boys and labourers in the Anglo-Egyptian Sudan: there were no toxic signs and no cases of sleeping sickness within three months of inoculation (Davey, 1948). The general routine in prophylactic inoculations has been to give the prophylactic drug to every second or third person coming for injection. Harding and Hutchinson (1948), however, have given prophylactic treatment to whole villages in Sierra Leone, as has McLetchie (1948) in Nigeria. It seems probable that injection of the population of whole villages in particular areas gives a more accurate picture of the results of prophylaxis, for when every second or third person is selected, the use of the prophylactic tends to reduce the rate of infection among the uninoculated controls, just as inoculating three-quarters of a community against smallpox will to some extent protect the remainder.

Earlier results of prophylactic injections are summarised by Mayer (1928). Working in a highly infected area in the French Moyen Congo, Arnaud (1929) gave suramin to 450 persons. Thirteen months after the injection the number of fresh infections showed a close correlation with the dose of suramin administered.

THE EFFECT OF DOSAGE ON THE PROPHYLACTIC ACTION OF SURAMIN (Arnaud, 1929)

Dose in gm. per kgm. of body weight.	Number of persons injected.	Number of new cases.	Percentage of new cases.
0·02 . . .	25	0	0
0·02 to 0·015 . .	57	4	7
0·015 to 0·010 . .	247	41	17
Less than 0·010. .	121	39	32

Fourche and Haveaux (1931) reported that of a population given 2 gm. of suramin in two doses only 0·09 per cent. had become infected thirteen months later, whereas of controls not given the drug, 4·1 per cent. had contracted sleeping sickness.

De Brauwere and Lisfranc (1931) found that three injections of suramin were preferable to five of tryparsamide, but to be effective the whole proceeding had to be repeated at intervals of six months. In a community of 621 persons in which twenty new cases of sleeping sickness were found in 1934 and twenty-one in 1935, Orlovitch (1937) gave suramin to almost the whole population, repeating the dose at intervals of three months. Examinations made on considerable numbers up to three months after the last injection failed to reveal any new cases, but four persons who had never received the drug became infected. In a long-standing but still active focus of sleeping sickness in the Belgian Congo, where new cases in 1942 numbered 3·69 per cent. of the population, Fain (1942) gave every member of the community 0·025 gm. of suramin per kgm. of body weight. Only twelve new cases of trypanosomiasis were found during the next nine months among 4,500 persons inoculated, as against 162 in the previous year. De Marqueissac (1932) reported somewhat unexpected results. Half the negative persons in three villages were treated with 0·02 gm. per kgm. of suramin ; from 82 to 123 days later 10 per cent. of treated and 8 per cent. of untreated persons were infected. In mining camps in Nigeria, McLetchie (1948) found that suramin gave protection for six but not for twelve weeks. There were certain toxic reactions in this community and Lester (1938, 1939, 1945), after a considerable experience of suramin, points out that with a dose of 1 gm. of suramin alarming collapse or even death may occur approximately once in 2,000 first injections. For this reason he raises the question whether it is reasonable to expose healthy persons to the risk of an injection. While there is evidence that suramin, because of its persistence in the blood stream, can protect persons from infection by trypanosomes for about three months, with a minimum of fifty-eight days, there are certain other drawbacks to its use, apart from toxicity. To perform intravenous inoculations on an entire population every three months necessitates very considerable organisation and a large staff ; it is also time-consuming (van Hoof *et al.*, 1940). Intramuscular injections are painful and are not readily submitted to while at least one case of gas gangrene is known to have followed this method of administration. As Hawking (1940) has pointed out the concentration of suramin in the blood following injection varies very considerably, and finally there is some evidence that suramin may give rise to cryptic infections, trypanosomes being present in the cerebrospinal fluid but absent from the blood and lymph nodes.

Prophylaxis with Aromatic Diamidines

The prophylactic action of stilbamidine, pentamide, and propamidine in the mouse was first studied by Launoy and Lagodsky (1940) in infections due to *T. brucei*. Launoy and Jeanpierre (1948) recorded similar experiments with pentamidine in animals infected with *T. equiperdum*. In mice, doses of 0·3 to 0·5 mgm. per 100 gm. three days before infection caused a totally refractory state in 20 per cent. of animals. Doses of 0·8 to 1·0 mgm. are necessary to ensure 60 to 100 per cent. of survivors if the drug is given for six days before infection. In rats a subcutaneous injection of 2 mgm. per 100 gm. completely protected for 191 days. In guinea-pigs a dose of 1·5 to 2·0 mgm. protects for about fifteen days (Launoy and Chaboud, 1948). Launoy and Lagodsky (1946a, b and c) obtained similar good results with pentamidine in rats infected with a number of different species of trypanosome.

The relation between the curative and the prophylactic doses giving protection for from twenty-five to thirty days was as follows (Launoy and Lagodsky, 1946c) :—

Infection.	Dose in mgm./100 gm. body weight.	
	Curative.	Protective.
T. brucei	0·5	2·0
T. equiperdum	0·7–0·9	2·0–2·5
T. evansi	7·0–>9·0	25·0

In the majority of cases pentamidine has been injected subcutaneously as a prophylactic. Launoy (1947) has shown that it may also be given prophylactically by mouth. Lomidine, the 2 : 2′-methylene-bis-hydroxynaphthoate of pentamidine base, is insoluble in water but is apparently absorbed from the intestinal tract. In rats, doses of 0·15 to 0·3 gm. per 100 gm. body weight were given orally and a heavy suspension of the Yaoundé strain of

T. gambiense was injected intraperitoneally. Whereas untreated rats all became infected in the usual way, those treated with lomidine by mouth remained free from infection for periods which varied up to about 285 days.

Experiments on the use of pentamide as a prophylactic were undertaken by van Hoof *et al.* (1944) in the Belgian Congo, it having been found that guinea-pigs given three injections of pentamidine, 0·002 gm. per kgm. of body weight, were rendered resistant to the bites of infected flies for 120 days. Human volunteers given 0·002 to 0·003 gm. per kgm. of body weight were resistant for ten to twelve months. Fulton (1944) investigated the prophylactic action of several aromatic diamidines in animals. Prophylactic activity appears to be proportional to curative action, but the resistance shown to repeated attempts at infection was, it is suggested, due to immunisation as the result of frequent injections of trypanosomes rather than to the drug itself. This question has been reinvestigated by van Hoof *et al.* (1946a and b), who state that one injection of pentamidine can protect a man for six months in the absence of any contact with infected flies; the prophylactic action of pentamidine is thus not necessarily due to premunition. In French West Africa prophylactic pentamidine is now being given as a routine and nearly 100,000 persons have been treated.

Prophylactic injections with pentamidine were begun on a considerable scale in the Kwango district of the Belgian Congo in 1942, as this was an area where 9 per cent. of the population was known to be infected; both pentamidine and propamidine were used (van Hoof *et al.*, 1944, 1946a and b; van Hoof, 1947; Claessens, 1946). With pentamidine isethionate the prophylactic dose is from 3 to 5 mgm. per kgm. of body weight. Of 721 Africans originally protected in 1942, none has become infected (van Hoof, 1947) and results on the whole are considered to have been extremely promising.

In Kongolo, 5,000 persons have been protected with pentamidine isethionate since November 1946: not a single case has occurred in those injected. In various foci in the Equatorial Province of Coquilhatville, 6,000 Africans have been given pentamidine, others were given suramin, while a third group remained as controls. Among those given pentamidine only two new cases were found ; among the controls the infection rate remained between 1·3 and 2·3 per cent. (van Hoof, 1947).

In Nigeria, according to McLetchie (1948), pentamidine isethionate is now given in a dose of 250 mgm. every four to five months to those working in mining camps. In two years there has been found no overt or cryptic infection among those given pentamidine, even up to a year previously. In the Plateau Province of Nigeria two-thirds of the miners are now regularly protected.

Harding and Hutchinson (1948) used pentamidine in Sierra Leone, in the Fuero area, where a somewhat atypical strain of trypanosome exists in that just over a third of the cases are negative to blood or lymph node examination on any one occasion. In view of the difficulty of assessing the value of a prophylactic in an area where many infected persons are almost certain to be given a prophylactic injection, a preliminary study was made of the effects of giving a single injection of suramin or pentamidine isethionate to persons already infected.

The table shows the results of a single prophylactic injection of suramin or pentamidine given seven months previously :—

Results of Prophylactic Injections after Seven Months

Drug.	Dosage.	Number treated.	Died.	Number observed.	Percentage relapsing in seven months.
Suramin .	1 gm.	26	0	22	27·2
Pentamidine isethionate	100 mgm.	17	0	16	35·0
,,	300 mgm.	24	0	23	
,,	375–500 mgm.	38	3	32	9·4

Thus even one prophylactic injection will cause trypanosomes to remain scanty or absent in the peripheral blood for as long as seven months.

Bearing these results in mind, the figures for prophylaxis given to the whole population are remarkable. They are shown in the table on p. 468 :—

RESULTS OF PROPHYLAXIS IN THE FUERO AREA, SIERRA LEONE
(Harding and Hutchinson, 1948)

Drug.	Dosage.	Number injected.	Number re-examined.	Percentage with trypanosomes in the blood.
Suramin . . .	1 gm.	483	437	1·4
Pentamidine isethionate	150–200 mgm.	692	549	0·7
,, ,,	375–400 mgm.	590	512	1·0
Total prophylaxis .	—	1,765	1,498	1·0
Controls—no prophylaxis . . .	—	518	471	5·7

These figures from an area where there was an unusual strain of trypanosome compare favourably with those from areas with more usual strains as shown in the table :—

Area.	1945 Sleeping' sickness incidence. (per cent.)	Drug.	Number injected.	Number of persons infected, 1946.
Mofindo .	4·0	Suramin 1·0 gm.	309	0
		2·0 gm.	213	1
		Pentamidine isethionate 100 mgm.	153	0
		150 mgm.	239	0
		350 mgm.	160	0
		Controls	548	2
Toli .	1·8	Suramin 1·0 gm.	80	0
		2·0 gm.	170	1
		Pentamidine isethionate, 150 mgm.	237	0
		300 mgm.	132	0
Kunda .	2·0	Controls	846	6
Ndakele .	2·3	,,	1,013	14

In the Oubangi-Chari District of French Equatorial Africa, 409 inhabitants of three villages were examined in June 1946 : 144 of these persons were already under observation after previous treatment : four new cases were discovered. The remaining 261 persons were given a single intramuscular injection of 4 mgm.

of pentamidine for children aged five to twelve years, and 5 mgm. for all others. The results are shown in the table, the index of new cases being determined, in accordance with the Brazzaville International Trypanosomiasis Conference 1948, as :

$$\frac{\text{Total number of new cases} \times 100}{\text{Total number examined less the number of old cases not cured.}}$$

CHEMOPROPHYLAXIS BY PENTAMIDINE IN OUBANGI-CHARI DISTRICT OF FRENCH EQUATORIAL AFRICA.
(Saleun and Chassain, 1948).

	Date of examination.	New cases.	Index of new cases. Per cent.
Before treatment .	1st Quarter 1945	26	5·7
	2nd ,, ,,	20	4·8
	3rd ,, ,,	4	1·09
	4th ,, ,,	10	3·2
	1st ,, 1946	13	4·06
	2nd ,, ,,	12	3·8
After treatment .	3rd ,, ,,	2	0·9
	4th ,, ,,	0	0
	Jan.-Feb. 1947	0	0

Similar results were obtained by Brun-Buisson *et al.* (1947) in French Guinea. In two areas 1,002 persons received 300 or 600 mgm. pentamidine per kgm. of body weight. No fresh cases of trypanosomiasis were seen in six months, but among 902 controls, who were untreated, there were nineteen cases.

Propamidine has also been extensively used in the Belgian Congo as a prophylactic. Claessens (1946) protected 159 Africans living in a highly infected area ; one patient was found with positive symptoms six months after the injection. van Hoof *et al.* (1947) also carried out prophylactic injections with propamidine. Eeraerts (1947) used propamidine in the Muzengo area, which had a new infection rate of 2 per cent. prior to prophylaxis. Similar injections were given in the Mwela-Zemba area, which had infection rates of 3·63 and 5·97 per cent. in two different localities, reaching at some points to from 28 to 49 per cent. Considerable numbers of tryparsamide-resistant cases were encountered, and in some villages as many as 100 per cent. of the patients were arsenic-resistant. Two injections of propamidine were given at monthly intervals to a total of 8,000 persons and for eighteen months thereafter there was not a single new case.

Fain and de Mulder (1948) similarly carried out investigations in two areas in the Belgian Congo. In the Bongons area, of 2,551 Africans, 2,133 were injected twice at an interval of six months whereas 418 remained as controls. In the ensuing two years there were twenty-three cases among the controls in contrast to one among those inoculated. In a second area, Kintswomo, the inhabitants of one village, Ngunu, were inoculated while twenty-two other villages were left as controls. Among the 285 persons inoculated there were during the next year, 1946, no cases, although in 1943, 1944 and 1945 there had been eighteen, four and forty-five respectively. In all the other villages the epidemic continued unabated. The doses of propamidine employed were 0·3 gm. for an adult male, 0·23 to 0·25 gm. for an adult female. Children received slightly larger doses than would be indicated by their body weight : pregnant women received only 0·1 gm.

A curious finding was that in the village of Ngunu, nine months after the last injections about 10 per cent. of those prophylactically injected showed cellular changes in the cerebrospinal fluid although trypanosomes could not be found either in the blood, lymph nodes or cerebrospinal fluid. Unfortunately the protein content of the cerebrospinal fluid was not studied. It is therefore impossible to be quite certain whether the increase in cell content of the cerebrospinal fluid was due to some other intercurrent infection such as a coccal skin condition or whether it was due to a cryptic infection which had preceded the date of the prophylactic injection.

In giving propamidine and pentamidine isethionate intramuscularly it is preferable to use a 1 in 25 solution. Toxic reactions have practically never occurred with pentamidine, but propamidine has been found occasionally to cause abortion in pregnant women.

The aromatic amidines are undoubtedly of considerable value in the prophylaxis of trypanosomiasis but they cannot be regarded as having an absolute preventive value. A dose of 5 mgm. per kgm.

of body weight is not always curative even in the earliest stage, and some patients must have already been infected at the time the survey is carried out. In addition, there may be variations in the rate of elimination or transformation of the drug. There is, however, already proof that when applied with adequate personnel and the necessary care pentamidine can bring about a complete eradication of the circulating parasites in man in a limited area. On the other hand, tsetse flies already infected and fed on animals injected with pentamide are not rendered free from trypanosomes nor is the cyclical development of the trypanosomes influenced. The great importance of pentamidine obviously lies not so much in the prophylaxis of the individual as in its potentiality for protecting a whole community by cutting off the source of infection for the tsetse fly.

Prophylaxis with other Compounds

Browning and Gulbransen (1934) found that, in addition to suramin, certain benzoylamino styryl quinoline compounds exhibit pronounced prophylactic action in mice. This protective power is due to the fact that when a watery solution is injected into animals subcutaneously a local deposit forms, accompanied by considerable reaction on the part of the surrounding connective tissues. The compounds are localised intracellularly, and after the subcutaneous injection of 1 ml. of a 1 in 200 or 1 in 250 solution of 2(*p*-acetylaminostyryl)-6(*p*-aminobenzoylaminoquinoline methoacetate) the drug still persists at the site of inoculation and still protects the animal from infection for almost a year. Though the amount of the drug present in the circulation at any one time is comparatively small, it is sufficient to prevent infection. Although striking prophylactic action is associated with the presence of *p*-aminobenzoyl, *p*-dimethylaminobenzoyl and *p*-acetylaminobenzoyl groups substituted in the 6—NH_2 group of the quinoline nucleus, the analogous trypanocidal substances in which the 6-position is occupied by —$N(CH_3)_2$ or —$NH.CO.CH_3$ exert only a brief prophylactic action. This can be correlated with the fact that after subcutaneous injection they are rapidly absorbed and rapidly excreted.

Mayer and Brousseau (1946) believe that the protective action of melaminylphenylstibonic acid, a quinquevalent antimalarial, in infected mice is due to immunity rather than chemoprophylaxis.

Very little has yet been done to evaluate drug prophylaxis in cattle. T-W-Fiennes (1948), however, has shown that it is possible to maintain a large herd of cattle in a tsetse-infested area by drug control with phenanthridinium compounds, the stock over a number of years becoming resistant or tolerant to the parasite. Similarly, subcurative doses of phenanthridinium compounds will convert fatal infections in cattle due to *T. congolense* or *T. vivax* into benign infections.

Wilde (1947) compared the prophylactic action of phenidium and stibophen : various dose-schedules were tested, phenidium being given as a single dose, once a month or every fourteen days after the finding of a positive blood film. Phenidium was found rather more effective than the antimony compound. No tests appear to have been carried out on the development of drug-fast strains as a result of chemoprophylaxis with phenanthridinium compounds.

Even more promising prophylactic experiments have been reported with antrycide salts in cattle by Curd and Davey (1949). Mice, rats and rabbits were first treated with the drug and after some weeks they were injected with *T. congolense*. In mice a single dose of 12·5 mgm. per kgm. of body weight provided protection for some weeks.

PROPHYLACTIC EXPERIMENTS IN MICE INFECTED WITH *T. congolense* (BUSIMBI STRAIN) USING ANTRYCIDE CHLORIDE. (Curd and Davey, 1949).

Dose (mgm./kgm.).	Interval between dosing and challenging in days.	Result.
25	14	10/10 P
12·5	14	8/8 P
5	14	4/11 P 7/11 I
25	28	8/8 P
12·5	28	8/8 P
5	28	8/8 P
12·5	42	2/6 P 4/6 I
5	42	8/8 I

P = complete protection,
I = delayed deaths.

Antrycide salts give considerable protection to cattle against subsequent infection with *T. congolense* and *T. vivax.* The duration of the protection extends to three or possibly six months.

Rollo *et al.* (1949) find that sodium *p*-melaminylphenylstibonate, the antimony analogue of melarsch oxide, protects mice for 41 weeks against *T. rhodesiense* in a dose of 0·16 mgm. per 20 gm,

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DRUG RESISTANCE

One of the most important problems with which chemotherapy is faced is the occurrence, among susceptible parasites treated by a drug, of strains which are no longer susceptible to the drug in the same concentration as heretofore. This heightened resistance to chemotherapeutic drugs is known as drug resistance or drug fastness. Although first described in relation to protozoa, it appears to be a generalised phenomenon, since it may occur with bacteria, viruses and possibly insects; certain races of house-fly are now being discovered which are resistant to the insecticide D.D.T. (King, 1948). Fixed tissue cells such as those of the kidney and liver may become resistant to the toxic action of uranium or chloroform (MacNider, 1936).

Drug resistance was first discovered by workers in Ehrlich's laboratory (Browning, 1907 ; Franke and Roehl, 1907 ; Ehrlich, 1907) soon after work had been begun on trypanosomes. It was noted that when parafuchsin (pararosaniline hydrochloride) was fed to mice infected with *T. brucei* the parasites soon disappeared from the peripheral blood stream ; after a week or two, however, parasites reappeared and further administration of the drug caused them to disappear again. This process could not be repeated indefinitely for after a time the parafuchsin produced less and less effect until finally a time was reached when the drug entirely failed to influence the parasites. On transferring the parasites to normal mice they were found to be still uninfluenced by parafuchsin and it became evident that they had undoubtedly acquired a heightened resistance to the drug. At about the same time atoxyl began to be used in the field against African sleeping sickness. Broden (1906) and Broden and Rodhain (1908) very soon detected the existance of strains of *T. gambiense* with a high degree of resistance to atoxyl. These fundamental observations were quickly followed by the discovery that strains of trypanosomes could be made resistant to a considerable number of drugs. In fact there are now few, if any, compounds with a chemotherapeutic action on trypanosomes against which drug-resistant strains are not known to exist.

The principal substances associated with resistant strains include :—

(1) Aniline dyes: parafuchsin, acriflavine (trypaflavine), dichloroparafuchsin (tryparosan).

(2) Aromatic compounds of arsenic : atoxyl, arsacetin, tryparsamide, reduced tryparsamide thioglycollate, halarsol, orsanine, 3-amino-4-hydroxyphenylarsonic acid (Fourneau 189), 3-hydroxymethyl-4-aminophenylarsonic acid (Fourneau 683) and 2-hydroxy-4-hydroxymethylphenylarsonic acid (Fourneau 722), neoarsphenamine, arsenophenylglycine, pyridone-arsonic derivatives (Schlossberger and Schüffner, 1934).

(3) Aromatic compounds of antimony : stibenyl, stibophen.

(4) Arsenoxides: amino- and amide-substituted phenyl arsenoxides.

(5) Non-aromatic compounds of antimony: tartar emetic.

(6) Suramin.

(7) Styryl quinoline derivatives: 2(*p*-acetylaminostyryl)-6-dimethylaminoquinoline methochloride and 2(*p*-aminostyryl)-6-acetylaminoquinoline methochloride (Browning, Cohen, Ellingworth and Gulbransen, 1929).

(8) Surfen C.

(9) Phenanthridinium compounds: phenidium and dimidium bromide (in cattle only but not in mice; Browning, 1949).

(10) Guanidines: synthalin (decamethylene diguanidine hydrochloride) (Schern and Artagaveytia-Allende, 1936; Browning, 1938).

(11) Diamidines: N-undecane 1:11-diamidine (Lourie and Yorke, 1938).

(12) Antrycide (Wilson, 1949).

(13) Serum.

Methods of Production. Three *in vivo* methods have been used for the production of resistant strains of trypanosomes.

(1) THE RELAPSE METHOD. This, the classical technique originally devised in Ehrlich's laboratory, consists in the administration to animals of a dose sufficient to remove trypanosomes from the blood temporarily but not sufficient to eradicate infection. When the relapse takes place, another dose is given, and so on, successive relapses being treated by slightly higher and higher doses as resistance develops. Since every relapse is preceded by antibody formation, trypanosomes acquiring drug resistance necessarily do so in the presence of considerable amounts of antibodies. Such antibodies may interfere with the appearance of drug resistance.

(2) THE SHORT-PASSAGE METHOD. Heavy infections are treated with very small doses of the drug and parasites are transferred to new hosts within the next four to eight hours.

Each succeeding passage is carried out in this same manner, the dosage being increased step by step. Thus the trypanosomes escape the influence of antibodies since they are passaged before the latter have time to appear.

(3) SPLENECTOMY. Animals are infected the day after splenectomy, daily treatment being started within the next twenty-four hours. Here again the aim is to minimise the influence of antibodies by removing one of the main supposed sources of immune bodies. In view of the supposed *rôle* of lymphocytes in the formation of immune bodies, the possibility of reducing the number of lymphocytes by exposure to X-rays or by inanition might be of value.

(4) SPONTANEOUS OCCURRENCE. It has been shown that a considerable degree of arsenic resistance may occur spontaneously (Morgenroth, 1924; Browning, 1931; Eagle and Magnuson, 1944). The resistant variants displayed the same characters of reduced affinity to drugs and chemical specificity as are shown by experimental arsenic-resistant strains.

(5) INDIRECT METHOD. A tartar-emetic resistant strain can be produced by treating an atoxyl-fast strain with two or three doses of tartar emetic (Mesnil and Brimont, 1908; Ehrlich, 1908).

The ease with which resistant strains are produced under laboratory conditions shows considerable variation. Yorke, Murgatroyd and Hawking (1932a) found that with atoxyl, acetarsol, tryparsamide, reduced tryparsamide thioglycollate, stibenyl and acriflavine highly resistant strains can be produced within four to eight weeks. After a single massive injection of tryparsamide in a cat infected with *T. annamense* the relapse strain which appeared in the blood stream three months later was found by Launoy (1937) to be highly resistant both to tryparsamide and acriflavine. Bovet and Montézin (1937), on the other hand, working with an old laboratory strain of *T. brucei*, failed to produce any recognisable degree of resistance on the part of the trypanosome either to atoxyl or orsanine with a single dose of atoxyl (1 mgm. per 20 gm. mouse). A single dose of orsanine, tryparsamide or 3-amino-4-hydroxyphenylarsonic acid failed to produce resistance either against itself or against any of these other compounds. A single dose of 3-hydroxymethyl-4-aminophenylarsonic acid (10 mgm. per 20 gm. mouse) produced great resistance both to itself and to orsanine, as also did a single dose of 2-hydroxy-4-hydroxymethylphenylarsonic acid.

Naito and Oka (1936) found that maximum resistance of a strain of *T. brucei* was reached against orsanine after thirteen passages in mice, but, in the case of neoarsphenamine and silver neoarsphenamine, not until the fiftieth passage.

The production of halarsol- and neoarsphenamine-fast strains requires, as a rule, about three months; the arseno-phenylglycine-fast strain develops still more slowly. Resistance to acriflavine develops to a maximum in about six weeks, some resistance being noticeable after fourteen days. The appearance of spontaneous resistance on the part of *T. equiperdum* against amino- and amide-substituted phenyl arsenoxides and their derivatives seems to have been quite sudden (Eagle and Magnuson, 1944).

To become resistant to undecane diamidine, although such strains can be obtained both in rabbit and mouse as well as *in vitro*, it is necessary to continue exposure to the drug for some considerable time (Lourie and Yorke, 1938).

Resistance against suramin develops very slowly and a suramin-resistant strain of *T. rhodesiense* may require more than twelve months to attain maximum resistance. Leupold (1925), using a strain of *T. brucei*, found that the maximum degree of suramin-resistance did not develop till after more than 100 passages. On the other hand, von Jancsó and von Jancsó (1935a, b) showed that a very considerable degree of suramin-fastness could be induced after twelve treatments in mice, provided that the activity of the reticulo-endothelial system was eliminated, first by splenectomy two to four hours after treatment, and secondly by the intravenous injection of colloidal copper three to four hours after the injection of suramin. It would thus seem that trypanosomes possess a capacity for becoming resistant to suramin, but in the normal animal this tendency is counteracted in some way by the intact reticulo-endothelial system.

Yorke, Murgatroyd and Hawking (1932a) drew attention to the fact that it is not easy to produce a strain of trypanosome which is resistant to tartar emetic. However, a strain which has been made atoxyl-fast in mice may be made resistant to tartar emetic by one or two doses of the latter drug. This fact has an important bearing on the occurrence of arsenic-resistant strains in the field.

Wilson (1949), working at Entebbe, in Uganda, showed that the soluble sulphate of antrycide cured *T. simiæ* in pigs in doses of 3 to 5 mgm. per kgm. of body weight. One pig infected with *T. simiæ* and treated with 2 mgm. per kgm. of the relatively insoluble chloride relapsed and attempts to cure infection with 5 mgm. per kgm, were then unsuccessful. In cattle also strains of *T. congolense* and *T. vivax* appear to have become resistant to antrycide with considerable rapidity ; after treatment they resist considerably higher doses. One such strain of *T. congolense* passaged in mice was not completely eradicated by 10 mgm. per kgm. of body weight of antrycide sulphate, whereas a field-strain obtained from an untreated cow in the same area was cured by 0·5 mgm. per kgm. of body weight of antrycide sulphate. The resistance had therefore increased at least twenty times. In some cases cattle reinfected some months after treatment with antrycide and retreated with curative doses have developed cryptic but nevertheless fatal infections. Should such cryptic infections or prolonged latent infections occur frequently after antrycide treatment an accurate estimation of the true duration of the prophylactic effect will be difficult if not impossible to assess. There is also some evidence to show that *T. vivax*, the other important cattle trypanosome, quickly develops an appreciable degree of drug resistance after treatment with antrycide. In animals which have relapsed or become reinfected after treatment with antrycide a dose of 5 mgm. per kgm. will not ensure sterilisation of the infection in every case, and may favour the development of drug resistance, and a dose in excess of 10 mgm. per kgm. can be toxic. Toxic symptoms develop quickly, the animal loses consciousness and death takes place two to six hours after treatment ; in other cases a severe gastro-enteritis develops forty-eight hours after treatment and death occurs in from six to twelve days. It is obvious that such results are not likely to recommend antrycide to the African cattle owner. Another complication reported by Wilson is that treatment with 1 mgm. per kgm. of dimidium bromide (phenanthridinium 1553) of relapse or reinfected strains of *T. congolense* following antrycide treatment has been unsuccessful : it appears, in fact, that strains resistant to antrycide are resistant also to curative doses of dimidium bromide. As dimidium bromide is one of the few other drugs which has given promising results in infections due to *T. congolense*, this is a serious drawback to the use of antrycide.

It will thus be seen that different compounds, even when closely related chemically, exhibit very different aptitudes for provoking drug resistance. In so far as the aromatic arsenicals are concerned, the production of resistance is in no way proportional to the amount of arsenic in the compound. This, however, is understandable, since the resistance is developed not against arsenic but against the non-arsenical substituted phenyl radical. The term " arsenic-resistance " is in fact a complete misnomer.

The more important strains of drug-fast trypanosomes may thus be arranged in the following groups :—

(*a*) Strongly resistant to the aromatic compounds of arsenic (except arsenophenylglycine) and of antimony ; slightly resistant to arsenophenylglycine ; sensitive to melarsen, butarsen (Eagle, 1945), tartar emetic, suramin, stilbamidine (Lourie and Yorke, 1938) and certain phenanthridinium compounds (Browning *et al.*, 1938), viz., atoxyl-fast, acetarsol-fast, tryparsamide-fast, reduced tryparsamide-fast, orsanine-fast, halarsol-fast, neoarsphenamine-fast, and acriflavine-fast strains.

(*b*) Resistant to arsenophenylglycine : moderately resistant to other aromatic arsenicals and antimonials ; sensitive to amino- and amide-substituted phenyl arsenoxides and their derivatives, to tartar emetic and to suramin : viz., arsenophenylglycine-fast strains.

(*c*) Resistant to amino- and amide-substituted phenyl arsenoxides and their derivatives (Yorke *et al.*, 1932a ; Eagle and Magnusan, 1944) ; sensitive to acid-substituted arsenoxides (King and Strangeways, 1942), to the unsubstituted phenyl arsenoxide (Hawking, 1937), and to phenyl arsenoxides with substituent groups such as $—CH_3$ or $—NO_2$, groups which do not significantly affect the toxicity or activity of the parent compound (Harris and Kahn, 1943) ; sensitive to tartar emetic and suramin, viz., amino- and amide-substituted phenyl arsenoxides-fast strains.

(*d*) Similar to Group (*a*) but resistant to tartar emetic : viz., tartar emetic-fast strain.

(*e*) Resistant to suramin ; sensitive to all other drugs : viz., suramin-fast strain.

(*f*) Resistant to amidine and guanidine compounds: sensitive to aromatic compounds of arsenic and antimony, acriflavine and suramin: viz., amidine and guanidine-fast strain.

(*g*) Resistant to antrycide and to dimidium bromide.

(*h*) Resistant to phenanthridinium compounds. No information is yet available whether such strains in cattle are also resistant to antrycide.

From these facts, as Yorke and his colleagues (1932b) and others have pointed out, it is possible to draw the following conclusions:—

(1) Resistance to one aromatic arsenical or antimonial compound implies resistance to other aromatic arsenicals and antimonials (Dubois, 1936), but this does not necessarily mean that a strain made resistant to one aromatic arsenical is identical with that made resistant to another. This is seen in the differences in degree of resistance exhibited by a tryparsamide-fast strain, an arsenophenylglycine strain or one made resistant to the monosodium salt of pyridone-3-arsonic acid (Schlossberger and Schüffner, 1934).

(2) A strain made resistant to an aromatic arsenical is sensitive to tartar emetic, whereas a strain made resistant to an aromatic antimonial is resistant to tartar emetic. This is probably due to the fact that stibenyl, like the other aromatic antimonial compounds undergoes decomposition when introduced into the animal body.

(3) Strains resistant to amino- and amide-substituted phenyl arsenoxides and their derivatives are not resistant to acid-substituted arsenoxides, to the unsubstituted phenyl arsenoxide or to phenyl arsenoxides with substituent groups such as $—CH_3$ or $—NO_2$.

(4) Strains made resistant to tryparsamide are fully sensitive to butarsen, to the disodium salt of 4-melaminylphenylarsonic acid and the corresponding arsenoxide (melarsen and melarsen oxide) (van Hoof, 1947; Williamson and Lourie, 1948).

(5) A strain of *T. congolense* resistant to antrycide is resistant also to dimidium bromide (phenanthridinium 1553) (Wilson, 1949).

During the second World War, when many persons taking mepacrine were serving in areas where trypanosomiasis is rife, it was feared that mepacrine might induce drug resistance to aromatic arsenicals. Such is not the case (Lourie and Collier, 1943); a strain of *T. rhodesiense* showed no tendency to become mepacrine-fast, and, of the few cases of trypanosomiasis among European army personnel who had been taking mepacrine prophylactically, none harboured a strain of *T. gambiense* resistant either to tryparsamide or suramin.

Although there is evidence to show that many, probably all, species of trypanosomes can become drug-fast, there is little evidence to prove whether there are differences in the ease with which different species or different strains of the same species become drug-fast.

The usual method of distinguishing between a drug-resistant and a normal strain of trypanosome is by determining the dose of the drug which will banish the trypanosomes from the blood stream for a given time. Schueler *et al.* (1947) developed an *in vitro* technique by determining the amount of the drug which would reduce the glucose utilisation by 50 per cent.

The Stability of Drug Resistance in Trypanosomes. In considering the stability of drug resistance in trypanosomes three questions require consideration :—

(1) The duration of the resistance.

(2) The persistence of the resistance when the trypanosomes are passaged through *Glossina*.

(3) The effect of passage from one animal species to another.

(1) The duration of drug resistance varies. A strain of *T. rhodesiense* made resistant to atoxyl was shown by Murgatroyd and Yorke (1937) to retain its characteristics for seven and a half years, and, later, by Fulton and Yorke (1941) for twelve and a half years, after passage through 1,528 mice. It was then still unaffected by the maximum tolerated dose of atoxyl, 0·8 mgm. per 20 gm. mouse, and its resistance to acetarsol, halarsol, stibenyl, and acriflavine was likewise unimpaired. Against suramin, tartar emetic and the aromatic diamidines it was just as sensitive as the parent strain.

Strains of *T. rhodesiense* maintained their resistance to tryparsamide and to acriflavine for seven and a half years, and a strain of *T. brucei* maintained its resistance unimpaired for a period of four years (Murgatroyd and Yorke, 1937). Browning (1908), however, noted that an atoxyl-resistant strain had lost its resistant character by the eighty-ninth passage. Robertson (1929) also reported a slow loss of resistance to acriflavine by a strain of *Bodo caudatus*. Piekarski (1949) found that a strain of *T. brucei* rendered resistant to euflavine (trypaflavine) lost its resistance in eight months. Schueler *et al.* (1947) showed that a strain of *T. equiperdum* rendered only slightly resistant to oxophenarsine lost this resistance after four passages in normal rats. A more highly resistant strain did not behave in the same way.

Suramin-resistant strains behaved somewhat differently. One strain lost its resistance in a period of four years and passage through 558 mice, the greatest decrease being in the first year. A second strain in rats lost a considerable amount of resistance and, after four to five months, no longer resisted 0·5 mgm. per 20 gm. mouse, whereas previously it had resisted a dose of 2 mgm. per 20 gm. mouse. During the ensuing three and a half years no further decrease in resistance occurred, though the minimal effective dose for the normal strain was 0·02 mgm. per 20 gm. mouse. A third strain rendered suramin-fast by enhancement from the second strain became resistant to 5 mgm. per 20 gm. mouse, and while sensitive to 10 mgm. even this enormous dose failed to cure. This strain maintained its resistance unimpaired for three years and five months; its sensitivity to aromatic arsenicals, to undecane diamidine and the aromatic diamidines remained that of the parent strain (Fulton and Yorke, 1941).

Two undecane diamidine-fast strains, one in mice, the other in rabbits, began to lose resistance after four months; after one year they reacted to small doses such as 0·05 mgm. per 20 gm. mouse, whereas they had originally resisted 0·25 mgm. per 20 gm. mouse. By the end of three years there was no trace of resistance.

A synthalin-fast strain prepared in rabbits and then transferred to mice behaved very much as the undecane-resistant strains. Originally 0·125 mgm. per 20 gm. mouse failed to cause the disappearance of the trypanosomes in half the mice; after four years all trace of resistance both to synthalin and to undecane-diamidine had been lost.

Attempts to influence or break down arsenic resistance by exposure to heat were unsuccessful (Janssens, 1936). Citron

(1931) claimed to have destroyed the resistance of an arsphenamine-fast strain of trypanosome by prolonged treatment of mice with sodium thiosulphate. Yorke, Murgatroyd and Hawking (1932b) failed to confirm these results.

(2) The question whether a drug-resistant strain is still drug-resistant after cyclical development in the tsetse was studied by Werbitzki (1910), Gonder (1911), Reichenow and Regendanz (1927), and Duke (1927). These experiments gave somewhat indefinite results, probably because the strains with which they were conducted were scarcely more resistant than the parent strains. Later, Yorke, Murgatroyd and Hawking (1933a, b) and Murgatroyd and Yorke (1937) reinvestigated the question and showed that a strain of *T. brucei* resistant to tryparsamide could be transmitted four times through *Glossina morsitans* in a period of four years without impairing its resistance. Passage of this tryparsamide-fast strain through *G. palpalis* was also obtained, though rather less readily (Murgatroyd, Yorke and Corson, 1937). Resistance to suramin survived at least one passage through *G. morsitans.* Lester (1932) also showed that a strain of *T. brucei* rendered serum-fast retained this character after at least one cyclical transmission through *G. tachinoides.* van Hoof (1947) believes that less resistant arsenic strains are more easily transmitted by *Glossina* than those which are very strongly resistant. He also considers that cyclical transmissibility of drug resistance is not absolute since some animals bitten by an infected tsetse may not show the drug-resistant strain but one with little or no resistance. The increasing prevalence of arsenic resistance in trypanosomes in certain areas must indicate the possibility of cyclical transmission by tsetses.

(3) The effect on drug resistance of passage from one animal species to another was first studied by Mesnil and Brimont (1908), who concluded that trypanosomes made resistant to atoxyl in the mouse may lose this characteristic when transferred to other hosts. This suggestion was supported by the work of Breinl and Nierenstein (1908) and others, but was contradicted by Yorke and Hawking (1932), who showed that a strain of *T. rhodesiense,* made resistant to atoxyl in the mouse, continued to manifest its full resistance when transferred to and passaged through rats or

rabbits: a strain made resistant to tryparsamide in rabbits likewise maintained its complete resistance when transferred and passaged in mice. *In vitro* experiments also yield results which indicate that the character of drug resistance is unmodified by transference of the trypanosome from one host to another: van Hoof (1947) has brought forward evidence to show that the host may have some influence on drug resistance. Resistant strains passaged in a number of guinea-pigs may show a reversion towards non-resistance in a particular guinea-pig while remaining fully resistant in others. Whereas in cattle *T. congolense* can become resistant to phenanthridinium compounds (Randalls and Laws, 1947), it has not yet been possible to produce drug-resistant forms in mice (Browning, 1949).

The Mechanism of Drug Resistance in Trypanosomes. Drug resistance in trypanosomes has been studied for more than thirty years and many of the general problems which are associated with drug resistance in other groups of parasites have been carefully investigated. In 1909 Ehrlich had noted that trypanosomes resistant to dyes remained unstained by these dyes. This was followed up by Levaditi (1909), who showed that arsenic-resistant trypanosomes fixed less arsenic than normal trypanosomes. The significance of these earlier studies was overlooked for twenty years till Yorke and his colleagues began to reinvestigate the problem. The first step in their experimental studies was to evolve a technique whereby suspensions of trypanosomes could be kept alive *in vitro* in undiminished numbers and with unlowered vitality for at least twenty-four hours at 37° C. (Yorke, Adams and Murgatroyd, 1929). Later Yorke and Murgatroyd (1930) and Yorke, Murgatroyd and Hawking (1931) showed that *in vitro* normal *T. rhodesiense* trypanosomes removed reduced tryparsamide from the surrounding medium whereas resistant ones did not. No investigations were made to determine whether removal was due to absorption of arsenic or to neutralisation. In 1932 Reiner, Leonard and Chao (1932a, b) found that *in vitro* at room temperature normal trypanosomes absorbed appreciable quantities of the tervalent arsenicals, neoarsphenamine, sulpharsphenamine, stabilarsan, and 3-amino-4-hydroxyphenylarsenoxide, the distribution of the arsenic being determined chemically: the quinquevalent atoxyl was not absorbed. Dead trypanosomes absorbed much more than living ones, but resistant trypanosomes bound rather less than normal ones. The differences were not thought to be sufficiently great to explain the mechanism of drug resistance, and in 1935 Pedlow and Reiner, in reinvestigating the problem at 37° C., were unable to detect any difference between the amount of arsenic fixed *in vitro* between arsenic-resistant and normal trypanosomes : *in vivo* arsenic-resistant organisms fixed a somewhat smaller quantity than normal trypanosomes. Hawking (1937) suggested that the strain of trypanosome used by these workers may not have been maximally resistant, rather strong concentrations of arsenic were used and in the majority of experiments the drug employed was neoarsphenamine, a compound which appears to be somewhat unsatisfactory for work of this kind. Hawking (1937), in addition, fully confirmed the results obtained by Yorke and his colleagues, using a chemical rather than a biological method of assay. The larger amount of arsenic bound by normal trypanosomes could not be due to the fact that a significant proportion of them had been damaged or killed by the arsenical since a difference in combining affinity was apparent even under conditions where the normal organisms had not been killed. Eagle and Magnuson (1944) similarly found that a strain of *T. equiperdum* which had become spontaneously resistant to amino- and amide-substituted phenyl arsenoxides bound less arsenic than normal trypanosomes from those arsenicals to which they were resistant, but that their affinity for phenyl arsenoxides to which they had not become resistant remained quantitatively unchanged. It should be noted that the varying susceptibility of normal trypanosomes to different arsenicals is also a direct function of their combining power with those compounds (Reiner, Leonard and Chao, 1932a, b; Eagle and Magnuson, 1944). So far as arsenicals are concerned, the statement by Ehrlich and Hata (1911) that " chemotherapeutic agents are not active unless bound " is entirely valid ; Fischl and Singer (1934b) showed that if normal trypanosomes are exposed *in vivo* to atoxyl, tryparsamide or neoarsphenamine they take up measurable quantities of arsenic. With the naturally resistant *T. lewisi* an appreciable quantity of arsenic was bound after exposure to arsenophenylglycine, a small amount after exposure to atoxyl and arsphenamine, and none after exposure to neoarsphenamine, solusalvarsan or sodium arsenite (Singer and Fischl, 1935). Similarly, normal trypanosomes absorb gold when exposed *in vivo* to 3 : 3′-diauromercapto-4 : 4′-disulpho-*s*-diphenylcarbamide, " sulfoharnstoff " (Fischl, Kotrba and Singer, 1934): absorption also occurs with *T. lewisi*, though the gold compounds are not trypanocidal. Hawking (1937) pointed out that absorption occurs very rapidly, being complete at 37° C. in a few minutes, though at 5° C. the process takes a few minutes longer to complete. Living resistant trypanosomes absorb little or none of the drug *in vitro* from low concentrations of such compounds as tryparsamide, halarsol or neoarsphenamine, but absorption occurs if stronger concentrations are used, or if the trypanosomes are dead. Similar differences between the behaviour of normal and resistant trypanosomes are observed when the parasites are exposed to reduced tryparsamide *in vivo*. Compounds to which atoxyl-resistant trypanosomes show no resistance, such as phenylarsenoxide, sodium arsenite, and tartar emetic, are absorbed to the same extent by the normal and resistant organisms. Arsenophenylglycine, which is rather less active on the resistant than on the normal trypanosomes, is absorbed in somewhat smaller amounts by the former. The quinquevalent tryparsamide, which is inactive *in vitro*, is not absorbed either by normal or atoxyl-resistant trypanosomes.

The action of the dyes of the acridine series, which chemotherapeutically resemble the aromatic arsenical compounds, was first studied by Roehl and Gulbransen (1909) and Gonder (1912), who observed the different behaviour of normal and resistant trypanosomes to vital staining. von Jancsó (1931 and 1932) demonstrated very conclusively that normal trypanosomes absorb acriflavine whereas resistant ones do not, since they are less photosensitive and their blepharoplasts do not take up the dye, an observation confirmed by Jadin (1932), Hasskó (1932), Fischl and Singer (1934b, 1935) and Pedlow and Reiner (1935). Singer and Fischl (1935) pointed out that when trypanosomes are continuously exposed to non-trypanocidal substances such as mepacrine or rivanol, they acquire the power of absorbing at least two and a half times the amounts taken up to begin with.

The evidence is thus strongly in favour of the view that trypanosomes resistant to aromatic compounds as well as to other substances escape injury because they fail to absorb the drug. The reason why resistant trypanosomes, and spirochætes also, absorb drugs less readily than normal trypanosomes is at present unknown. There is a possibility that the change may be associated with a loss, or more probably with a modification of specific receptors.

It is realised that the trypanosome must be furnished with a number of such specific receptor groups in view of the fact that resistance developed against one group of compounds does not produce resistance against other groups. At least four different groups of receptors can be distinguished. Schueler (1947) has suggested that drug resistance in trypanosomes may be resistance only to groups having a certain polarity, the actual resistance-acquiring process involving a shift in the isoelectric points of some of the proteins of the trypanosomes. By means of the method of Pischinger (1925) for finding the isoelectric points of the constituent parts of cells through their staining reactions with acidic and basic dyes over various *p*H ranges, Schueler (1947) has in fact revealed differences in the staining of strains of normal and drug-resistant trypanosomes of the same species, thus indicating possible differences in the isoelectric points of their constituent receptors. This change in isoelectric points would offer an explanation of why trypanosomes when made resistant to a given amino- or amide-substituted phenyl arsenoxide, as well as certain basic dyes, are resistant to other basic-substituted but not to neutral-substituted phenyl arsenoxides, the mechanism by which trypanosomes acquire resistance involving resistance to basic or acidic groups on the phenyl arsenoxide molecule and not to the arsenoxide groups.

King and Strangeways (1942) and King (1943) have attempted to explain the anomalous action of arsenophenylglycine and of unsubstituted phenylarsenoxide on trypanosomes resistant to aromatic arsenicals on somewhat different lines. Substances such as arsenophenylglycine containing carboxyl groups form readily soluble, highly ionised neutral salts and their ions do not easily leave the water-phase : hence their low trypanocidal power.

Highly active compounds which are equally effective against normal trypanosomes and those made resistant to aromatic arsenicals are usually devoid of hydrophilic groups and may be more easily transported within the cell by virtue of the resulting solubility in lipids. Compounds which are relatively inactive usually have polar groups and may therefore be waylaid by adsorption on polar surfaces on their way to their site of action. The active arsenoxides, on the other hand, would orient themselves at a lipid-water interface so that the phenyl or corresponding group is in lipid and the arsenoxide in the water phase. Such active compounds are thus easily mobilised at the site of action in the trypanosome. One difficulty with this theory, as pointed out by Eagle and Magnuson (1944), is that many compounds with hydrophilic substituents are highly active. In addition, the relatively inactive compounds are not bound by the trypanosome as they should be were they waylaid by adsorption short of the site of action. Similarly the resistant strain binds less of those arsenicals to which it is resistant and normal amounts of those to which it is normally susceptible.

Phenyl arsenoxides, in general, are thus active to the degree in which they are bound by the cell and the enormous differences in activity cannot easily be ascribed to their varying distribution on or within, the cell.

It is possible that there is a single explanation of the different susceptibilities of normal trypanosomes to different phenyl arsenoxides and the generally decreased susceptibility of an arsenic-resistant strain to, for instance, amino- and amide-substituted compounds. Intrinsically all phenyl arsenoxides may be equally active in that they have identical affinities for the receptor groups within a given cell species. The varying activity of different compounds may be determined simply by the degree to which substituents other than the —AsO group affect the penetration of the cell by the compound. Similarly, a genetic or adaptative change in the cell surface could well modify the ease with which certain substituted phenyl arsenoxides pass into the interior of the cell, without, however, affecting the permeability of compounds with other types of substituent groups.

One other possibility remains to be considered. Hawking

(1937) has suggested that the receptor groups for arsenic in a resistant strain are modified in the sense that they have a diminished affinity for tervalent arsenicals carrying certain substituent groups, while they maintain their affinity for such compounds as unsubstituted phenyl arsenoxide, which acts equally on normal and resistant trypanosomes. Because of an apparent correlation between the chemotherapeutic indices of a series of arsenicals (ratio of toxicity/trypanocidal activity) and their " resistance factor " (susceptibility of resistant strain/susceptibility of normal strain), Hawking has further suggested that the receptors in resistant organisms more nearly resemble those of the tissues of the host. However, in a large series of amino- and amide-substituted phenyl arsenoxides tested in this respect, Eagle and Magnuson (1944) have found no correlation between the toxicity of the compounds in mice and rabbits and their trypanocidal activity against the resistant strain, or between their chemotherapeutic index and their " resistance factor."

Efforts to link up arsenic resistance with a change in the sulphydryl groups have not yet been successful. An attempt was made by Harvey (1948) to determine the sulphydryl and disulphide content of normal and arsenic-resistant trypanosomes in view of the suggestion that the amount of tripeptide glutathione, which, in the reduced form, contains the —SH group, might play a part in the phenomenon of arsenic resistance in trypanosomes. A quantitative estimation of sulphydryl and disulphide groups, the latter capable of yielding the former on reduction, in normal and arsenical-resistant strains of *T. equiperdum* and *T. hippicum* showed that, when using the method of Mirsky and Anson (1935), the different values for —SH groups were not significant. The normal strain of *T. equiperdum*, however, contained an excess of disulphide groups, as contrasted with the resistant strain, whereas in the case of *T. hippicum*, normal and resistant, the reverse was the case. There is thus no indication of a definite relation between the actual and potential —SH content of the trypanosomes and arsenic resistance. Hence no explanation is forthcoming on these lines as to why certain arsenicals with an acidic side-chain are effective against trypanosomes resistant to arsenicals without such a side-chain.

There is, however, one morphological change which has been noted in trypanosomes during the development of resistance to acridine and oxazine dyes, a possible disappearance of the parabasal body or blepharoplast (Werbitzki, 1910; Kudicke, 1911; Laveran and Roudsky, 1911). Disappearance of the blepharoplast is not necessarily associated with increased resistance since spontaneous disappearance of the blepharoplast is known to occur. Leupold (1925) found that in arsenic-resistant trypanosomes the blepharoplast cannot be destroyed by acriflavine, as is the case with normal trypanosomes. If the trypanosomes lose their resistance the blepharoplast does not reappear (Piekarski, 1949). The blepharoplast of resistant trypanosomes, if it does not disappear, does not take up the dye.

The Genetic Mechanism of Drug Resistance. There is still considerable uncertainty as to the exact mechanism whereby drug resistance occurs. The evidence that it is always the direct result of environmental change, an example of Lamarckian inheritance, may be dismissed in view of the well-authenticated instances of spontaneous acquirement of resistance in the absence of treatment by a specific drug. Barlovatz (1933) and van Hoof (1947) believe that such spontaneous variation is constantly occurring among trypanosomes under natural conditions. In areas where there has been extensive use of arsenotherapy only those trypanosomes with a considerable degree of resistance would be able to survive. On the other hand, in the laboratory a very high degree of resistance can be built up. Yorke (1934), for instance, has shown that the concentrations of reduced tryparsamide necessary to kill normal and resistant *T. rhodesiense* are 0·04 and 10 parts per million respectively, a 250-fold difference.

Both in the laboratory and in the field, however, the mechanism would appear to be essentially the same; a variable population is subjected over a number of generations to a process of selection. In the laboratory the selective process is more intense. If, for instance, the range of drug fastness of a normal strain to an aromatic arsenical is represented as − 10 to + 10, the highest concentration of the drug which just fails to destroy them all would then allow the survival of trypanosomes with a resistance of + 10. These trypanosomes, given the same capacity to vary, would show a variation of from 0 to 20 ; in the next generation from 10 to 30, and so on, till only highly resistant trypanosomes eventually remained. The production of a drug-fast strain of trypanosomes is thus in fact analogous to the gradual adaptation of free-living organisms to an increase of temperature. Such adaptation occurs in a series of steps which are dependent on the successive appearance of mutants capable of living at progressively higher temperatures.

The further question remains whether drug resistance is due to a true chromosome mutation or whether it is of the nature of a " Dauermodifikation," due to changes in the cytoplasm. The occurrence of spontaneous drug-resistant trypanosomes and the frequent persistence of drug resistance for long periods without further exposure to the drug suggests that the change is a true genetic mutation. On the other hand there are instances, as in that reported by Schueler *et al.* (1947), where a slight degree of resistance to oxophenarsine was rapidly lost after four passages in rats.

In the case of bacteria it is suggested that either a variation may begin as a " Dauermodifikation " and, later, as the selective process continues, this may be converted into a chromosomal mutation, or a true chromosomal mutation may occur immediately. Similar changes may take place in trypanosomes. So far little has been done in trypanosomes to study enzyme variation and adaptation, a study which, in the case of bacteria, has thrown considerable light on the mechanism of drug resistance (Hinshelwood, 1946 ; Stephenson, 1949). Whether or not the drug resistance is primarily induced by the aromatic arsenical is still unknown, in other words, whether adaptation occurs. The difficulty of producing a strain resistant to tartar emetic, except in one which has been made resistant to arsenic, suggests that some change, either a " Dauermodifikation " or a mutation, is first necessary and the multiplication of this modified strain is then encouraged by the process of selection to which it is exposed.

In view of the work which has been carried out on drug resistance in bacteria, much further work is required on the same phenomenon in protozoa. Thus it would be of considerable interest to determine whether in trypanosomes, as in bacteria, the production of resistant forms by certain drugs is associated with adsorption of the drugs by a specific $H_2N-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-SH$ group rather than with a rapid reaction with an —SH group (Klimek *et al.*, 1948).

In the case of bacteria it has been claimed that growing penicillin-sensitive and penicillin-resistant organisms together may cause the penicillin-resistant organisms to become penicillin-sensitive (Pandalai and George, 1947 ; Voureka, 1948 ; Winner, 1948 ; George and Pandalai, 1949). It is suggested that a nucleic acid component extracted from penicillin-sensitive organisms reconverts the resistant organisms to sensitivity. It is possible that resistant trypanosomes might similarly be reconverted to sensitivity.

The Practical Importance of Drug Resistance. The practical importance of drug resistance lies in the fact that in many areas in Africa patients in increasing numbers are being infected with strains of trypanosomes that are resistant to tryparsamide. The areas where drug resistance is most common are Kwango, Kasai and round Leopoldville, in the Belgian Congo (Eerarets, 1946 ; van Hoof, 1947), Kordie, in the Ivory Coast, and the northern part of Togoland (Lapeyssonnie, 1948) and in the region of the Niger. Before 1938, in the Leopoldville area, only 7 per cent. of patients harboured trypanosomes which were arsenic resistant. From 1940 to 1945 about 50 per cent. of patients had arsenic-resistant trypanosomes, and by 1947 almost 100 per cent. of the trypanosomes were resistant (van Hoof, 1928, 1947 ; van Hoof *et al.*, 1938 ; Pearce, 1930 ; Spyrou, 1933). In French West Africa resistance is said to be restricted to certain villages. Lester (1933) found that arsenic resistance was not uncommon in a few areas in Nigeria. Harding (1945) records that eighteen months after treatment with up to 30 gm. of tryparsamide nearly half of a group of patients from the Bauchi Plateau in Nigeria were either dead or still infected.

Now that suramin is being more widely used, it is possible that arsenic resistance may be decreasing (McLetchie, 1948). Resistance to suramin itself is rare. One or two instances have been noted in Nigeria, where trypanosomes were still present on puncture of the lymph nodes after a standard treatment with suramin and tryparsamide, and in the Kwango and Kasai areas of the Belgian Congo strains resistant to suramin, tryparsamide and antimonials have been encountered (van Hoof *et al.*, 1938). It seems certain that the incidence of drug fastness is correlated with the methods of mass treatment employed. In French West Africa resistance is said to be restricted to villages where incomplete arsenical treatment was given in the years before the creation of the Sleeping Sickness Service. On the Bauchi Plateau, in Nigeria, irregular arsenic treatment had been carried out previously in areas where there was later a high incidence of resistant cases. In the Belgian Congo also the areas where sleeping sickness campaigns have been carried on longest are precisely those where drug resistance is most common. The combined use of antimony and arsenic by Belgian workers may have something to do with the high incidence of resistance in the Congo. Such a view is not accepted by van Hoof (1947), who does not believe that precisely the same mechanism is at work in the field as in the laboratory. van Hoof (1947), is of the opinion that mass treatment merely eliminates all trypanosomes with a low resistance to arsenic, leaving those with a higher degree of resistance to survive. There is also the possibility that, as a result of mass prophylaxis, trypanosomes may be exposed for considerable periods to sublethal doses of the prophylactic drug. Thus drug resistance may follow in areas where mass prophylaxis has been practised.

Up to the present, infections with arsenic-resistant trypanosomes have presented a very serious problem, unless they could be adequately treated with pentamidine before the central nervous system had been invaded. If the central nervous system had been attacked the individual was doomed though his blood stream could be sterilised with pentamide or, as van Hoof (1947) has shown, by means of melarsen oxide. Butarsen is ineffective in treating patients with secondary symptoms, and until recently it was thought that melarsen oxide was similarly useless. Friedheim (1948), however, has now reported that with large doses of melarsen oxide it is possible to treat successfully patients with involvement of the central nervous system. In view of the findings by van Hoof (1947) in the field, and by Williamson and Lourie (1948) in the laboratory, that tryparsamide-resistant strains are susceptible to melarsen oxide, this drug may be destined to play a large part in overcoming the menace of arsenic resistance in trypanosomes.

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CHEMOTHERAPEUTIC INTERFERENCE

In view of the fact that drug resistance is associated with a decreased power of absorption of the drug by the resistant trypanosomes, the phenomenon brought to light by Browning and Gulbransen (1922), and termed " therapeutic interference," takes on a new interest. It was found that when mice are fed with parafuchsin and are subsequently inoculated with a strain of trypanosome resistant to parafuchsin, the therapeutic action of trypaflavine is greatly reduced. These observations were confirmed by Schnitzer (1926), who showed that to demonstrate interference it is not necessary to employ trypanosomes which are resistant to parafuchsin. Schnitzer and Rosenberg (1926 and 1927) and Schnitzer and Silberstein (1926) in addition found that parafuchsin injected along with certain compounds, or preferably a few hours before, interfered with the chemotherapeutic action of trypaflavine, acetarsol, arsphenamine and tartar emetic, while pyoktanin (a mixture of penta- and hexamethyltriaminotriphenylmethane hydrochloride) interfered with the action of trypaflavine, acetarsol, arsphenamine, and tartar emetic but not with tryparosan (Schnitzer and Silberstein, 1927). Browning *et al.* (1931) showed that in the production of certain quaternary salts of styrylquinolines the methods of preparation may lead to the formation of isomerides, which are not only less trypanocidal, but interfere with the chemotherapeutic action of the actively trypanocidal compound. Fischl and Fischl (1934) observed an interference effect on the part of ascorbic acid with tartar emetic, arsenophenylglycine, *m*-amino-*p*-oxyphenylarsenoxide, and trypaflavine. Hasskó (1935) found that preliminary injections of methyl violet, ethyl violet, or pyoktanin greatly lessened the capacity of the trypanosomes to absorb acriflavine, while a preliminary injection of trypan red or trypan blue almost entirely prevented the absorption of acriflavine by the trypanosomes. von Jancsó (1931) showed that, after a preliminary injection of parafuchsin, trypaflavine did not render the trypanosomes photosensitive to the same degree as untreated trypanosomes. These experiments demonstrate that the phenomenon of chemotherapeutic interference is associated, not with any change in the tissues of the host, but, as in the case of drug resistance, with decreased absorption by the parasites. Wright and Hirschfelder (1930) have in fact shown that the chemotherapeutic interference associated with trypanosomes can be duplicated *in vitro* in cultures of yeasts. Acriflavine interferes with the carbon dioxide production of yeasts, but when the yeast cells are stained by methyl violet or brilliant green in solutions too weak to affect carbon dioxide production, they become less sensitive to the action of acriflavine, while yeasts stained with dilute solutions of acriflavine are less sensitive to methyl violet and brilliant green. The reaction appears to be due to adsorption of the first dye on the surface of the cell interfering with adsorption of the second dye.

Chemotherapeutic interference is, however, similar in certain ways to the phenomenon described by McKinley (1929), Thung (1931) and Salaman (1933), who found that in plants the injection of a feebly pathogenic strain of a virus protein protected the plants against the subsequent injection of a highly pathogenic strain of the same virus protein. Findlay and MacCallum (1937), and Dalldorf, Douglass and Robinson (1938), showed that animal viruses of low pathogenicity may on occasions protect against other antigenically distinct animal viruses of high pathogenicity.

It is obvious that, though in certain cases closely allied substances may exhibit the interference phenomenon, in others there is no obvious chemical relationship between the interfering substance and the compounds with whose action it interferes. In order to explain chemotherapeutic interference von Jancsó and von Jancsó (1936) have advanced the theory that the combinations of drugs which produce interference are only those which form thermodynamically reversible redox systems, the redox potential of which belongs to a definite potential range. The interference action is accordingly a characteristic attribute of electro-active bodies which readily store up and give off electrons. Thus in a series of twenty quinonoid redox dyes with graduated normal potentials, the activity varies gradually with the value of the normal potential. The weakly positive and negative dyes between the potential points of the redox scale + 0·115 and — 0·060 volt (pH 7), namely toluylene blue, thionin, cresyl blue, gallocyanin, toluidine blue, azur, methylene blue, pyocyanin, janus green, and æthylcapri blue, all exhibit a pronounced interference action, A distinct optimum is reached at + 0·011 volt: toluidine blue and especially azur I inhibit the action of even large doses of arsenoxide and stibophen. Eight dyes, the normal potential of which is below —0·060, were inactive. Ascorbic acid exerts a strong interference action against stibophen. This is explained by the fact that ascorbic acid forms a reversible redox system. The interference of such dyes as toluidine blue, cresyl blue, janus green and azur I on the trypanocidal action of arsenoxide was also confirmed by *in vitro* experiments, the action, for instance, of a 1 : 30,000 solution of arsenoxide, which normally killed trypanosomes in one or two minutes, was so far inhibited by the dyes that the parasites were still actively motile after fifteen or twenty minutes. von Jancsó and von Jancsó believe that the dyes which interfere with the action of tervalent arsenicals and antimonials act as accessory respiratory catalysts and prevent damage to the respiratory system of the trypanosomes. This suggestion receives support from the work of Scheff and Hasskó (1936), who found that trypanosomes which had been protected by chemotherapeutic interference showed no decrease in oxygen or sugar consumption as compared with trypanosomes treated only by the toxic drug.

Potassium hexatantalate probably acts by inhibiting tissue defences.

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TREATMENT OF HUMAN TRYPANOSOMIASIS

Trypanosoma gambiense Infections

Although much less work has been carried out on trypanosomes than on plasmodia, nevertheless it has long been recognised that there are different strains of *T. gambiense*. The ease with which some strains are maintained in laboratory animals and the difficulties of passaging others are well known. In some areas, but not in others, strains exhibit a very high tendency to invade the nervous system of man and animals (Stefanopoulo and Étévé, 1943 ; Roubaud, 1944 ; Roubaud *et al.*, 1944). It is also the general opinion that strains show different responses to chemotherapeutic drugs : strains, for instance, from Nigeria are thought to be more readily controlled by chemotherapeutic drugs than those from the Gold Coast, a suggestion which was confirmed during the treatment of African soldiers in the second World War. In Nigeria the routine treatment has been first an injection of 0·2 gm. suramin, followed by three injections of suramin 1 gm., and then by five injections of tryparsamide 2 gm. at intervals of five days : the first small injection of suramin is given to test for sensitivity. The same course was, and is, used in Sierra Leone and the Gambia. In Portuguese Guinea the first dose of suramin is usually 0·5 gm., followed three days later by 1 gm. and thereafter 1 gm. weekly to a total of 7·5 gm. Tryparsamide is given in doses of 2 or 3 gm. weekly for ten weeks (Pinto, 1947). Within the past few years a new technique has been applied in Nigeria and an analysis of the results suggests that it gives better results while reducing the time necessary for the whole course. Suramin is mixed with tryparsamide just before use, so that each injection contains 1 gm. of suramin and 2 gm. of tryparsamide. Three injections of the mixture are given, followed by two doses of tryparsamide 2 gm. alone. The injections are given at intervals of five days, with the result that the time required for this amended course is only twenty-five days as compared with forty days for the older standard course. This shortened course has also been tested in the Belgian Congo, but there it appeared to produce a somewhat alarmingly high proportion of optic lesions. In many areas, however, suramin-tryparsamide mixture (0·5 gm. suramin, 1·5 gm. tryparsamide) is being extensively used and synergistic treatment has been favoured by French workers for some years, especially in chronic cases (Sicé, 1937). A course of twenty injections, with four days between each injection, is given to patients in the second stage who have not responded to tryparsamide (Lapeyssonie, 1947). In the Belgian Congo it is now customary to give twelve weekly injections of tryparsamide (0·04 gm. per kgm.) followed by five weekly injections of suramin (0·02 gm. per kgm.) combined with tartar emetic in a dose of 0·0014 gm. per kgm. of body weight. The addition of tartar emetic is possibly a danger, since it may aid the appearance of arsenic-resistant strains of trypanosomes.

Death rates with standard combinations of suramin and tryparsamide vary very considerably, being much higher in Sierra Leone, among poorly nourished populations (Harding, 1945), than in Nigeria where, according to McLetchie (1948), deaths during treatment rarely exceed 1 per cent., blindness is less than 1 per 1,000, and exfoliative dermatitis is extremely rare. To reduce the risk of optic lesions, the eyesight should be examined before each injection of tryparsamide. A simple test for visual acuity is to pick up a pin from the table.

It is now generally agreed that pentamidine is as effective as suramin in the treatment of early cases, but its advantages are not sufficiently great for it to supersede suramin, especially when routine mass treatments are based on schedules involving five-day intervals for injections. Pentamidine can, however, be given daily, and if the intensive short course of pentamidine and tryparsamide, occupying seven to ten days, is employed, there is a considerable saving of time. For small groups of mild cases in remote areas difficult of access, the following course has been found satisfactory in Nigeria (McLetchie, 1948): pentamidine isethionate 100 mgm. on the first day, followed by 200 mgm. daily till the seventh day, together with 6 to 9 gm. of tryparsamide in the same seven to ten days. It must, however, be remembered that in a peasant community engaged in farming and trading at markets it is less disorganising to walk several miles once a week for some weeks to a treatment centre than to walk the same distance daily for seven to ten consecutive days. Earlier experiences with melarsen oxide suggested that it was of little value except in early cases. Friedheim (1948), however, gave 1·5 mgm. per kgm. of body weight intravenously, two series of seven daily injections being separated by a rest-period of one month. If his claims are confirmed, melarsen oxide will have a wide use, since it can be employed in tryparsamide-resistant cases.

The cure rates for *T. gambiense* infections vary somewhat but have shown considerable improvement in recent years. Harding (1945) reports 93·5 per cent. cures from Sierra Leone, McLetchie (1948) 80 to 95 per cent. in different areas in Nigeria.

Assessment of Treatment

In attempting to assess the value of a chemotherapeutic regime it is necessary to take into account :—

(1) Clinical symptoms.
(2) The protein content of the cerebrospinal fluid.
(3) The cell count in the cerebrospinal fluid.
(4) The blood sedimentation rate.

Clinical Cure. Spontaneous remissions and apparent cure may very occasionally occur without the aid of specific drugs. On the other hand, a considerable period free from all symptoms may elapse between the infecting bite and the development of nervous symptoms as witness the occurrence of trypanosomiasis in African soldiers stationed in India and Burma. Davey (1948) cites an instance from French West Africa where the infection appears to have remained latent for more than four years in the absence of prophylactic drugs. Mild cases undoubtedly do occasionally occur. Todd (1924), for instance, described survival for from nine to thirteen years in eight untreated cases. McLetchie (1948) similarly observed cases in Ilorin, Nigeria, where slight symptoms persisted for between five and eight years.

Slight differences in the mental states of Africans are often extremely difficult for European medical officers to determine : they are, however, readily appreciated by relations and friends. Where there is mental improvement the patient almost invariably becomes cleaner and, with increased appetite, scabies and the symptoms of multiple vitamin deficiency tend to disappear. In males, the return of potency is a characteristic change for the better; in females the return of menstruation and pregnancy are also good signs. The twins named by a proud and cured mother "Antrypol" and "Tryparsamide," are well known in the Gold Coast.

Cellular Content of the Cerebrospinal Fluid. Very soon after the discovery of trypanosomes in the cerebrospinal fluid in cases of sleeping sickness it was recognised that an examination of the cerebrospinal fluid was of importance, not only for diagnosis but for prognosis, since a return to normal in the cell content and in the total protein indicates that the patient is on the way to recovery. The presence or absence of trypanosomes in the cerebrospinal fluid is by itself valueless. Harding (1945), for instance, found trypanosomes in only four instances (twice in lymph node juice, once in blood and once in cerebrospinal fluid) among 1,732 patients re-examined subsequent to treatment. The true incidence of treatment failures is certainly higher than is suggested by these figures.

When the central nervous system is involved in trypanosomiasis there almost always occurs an increase in the cell content, this increase preceding the increase in total protein in the cerebrospinal fluid. The same sequence occurs in a relapse following drug treatment. In the majority of cases, after treatment with tryparsamide, the cell count falls to below 5 cells per ml. In a minority of cases, however, the cell count remains slightly raised for three or four years after treatment, even though there is every indication that the patient is cured. For this reason some workers adopt 10 cells per ml. as the limit of normality in assessing the cure rate, but the International Committee on Trypanosomiasis, meeting at Brazzaville in February, 1948, reported that 3 cells per ml. should be taken as the upper limit of normality. Many observers have noted that, after five or six injections of tryparsamide, the number of cells in the cerebrospinal fluid may first increase, decreasing almost to normal only after the twelfth injection (Péllissier, 1946).

It is still unknown why it is oftener easy to cure a patient with a very high cell content and a total protein content of 60 to 70 mgm. per 100 ml. than a patient with a low cell content (50 to 100 cells per ml.) and a higher protein content of 90 to 100 mgm. per 100 ml.

The cell content of the cerebrospinal fluid in treated patients suffering from trypanosomiasis may be influenced by other intercurrent infections. A diffuse septic condition of the skin may, it is said, cause a rise in the number of cells in the cerebrospinal fluid. Yaws has also been incriminated, and, in many communities suffering from sleeping sickness, yaws also is very prevalent. Harding (1945) examined the cerebrospinal fluids of twenty persons with no history of trypanosomiasis from such a community; in nineteen there were five cells or less per ml. and in only one was the cell content as high as 6 or 7 cells per ml. In Northern Nigeria where, in addition to trypanosomiasis, syphilis of the central nervous system is by no means uncommon, there is often difficulty in distinguishing between the two conditions. A certain number of patients with syphilis of the central nervous system are, in the course of mass surveys, almost certainly treated as cases of trypanosomiasis, but as tryparsamide is of value in both conditions probably little or no harm is done. Though the presence of the morula cells of Mott in the cerebrospinal fluid is not absolutely specific, it is highly suggestive of trypanosomiasis.

It is still uncertain whether the cell count or the protein content of the cerebrospinal fluid is the better guide to prognosis. Most French workers believe that the total protein content is the more accurate: Harding (1945), on the other hand, believes that the cell content is the more valuable. Of 917 cerebrospinal fluids examined for cells and total protein at a final survey, seventy-one were abnormal in that they showed more than 10 cells per ml. or protein about 35 mgm. per ml., or both these features, but only seven of the cases showed protein above 35 mgm. per ml. combined with a cell count of ten or less. The omission of protein estimation would therefore have entailed an error of about 10 per cent., but in calculating total numbers of patients in whom treatment failed, including those who were dead or blind, the error would be no more than about 2 per cent. Before a patient can be said to be cured it is essential that repeated examinations should be made over a period of at least fifteen months. Friedheim (1948) finds that after melarsen oxide it may take nine months for the cell count to return to normal.

Protein Content of the Cerebrospinal Fluid. The most accurate method for estimating total protein in the cerebrospinal fluid is undoubtedly the micro-Kjeldahl, but this is impracticable for routine work in the field. The colorimetric method of Wu and Ling (1927) is also very accurate ; it requires 0·1 ml. of cerebrospinal fluid and a photoelectric colorimeter or photometer for readings. As a routine, the two main methods used have been turbidimetric (or opacity) techniques and precipitation methods. Of the precipitation methods used, that of Sicard and Cantaloube (1916) enjoys considerable popularity. The proteins are precipitated in a standard tube and after standing for five hours the precipitate is read from the calibrations on the tube. The first division on the tube indicates a total protein of 22 mgm. per 100 ml., the second division a protein content of 40 mgm. per 100 ml. Sicard and Cantaloube consider that any cerebrospinal fluid giving a precipitate whose height is more than one and a half divisions (above 30 mgm. per 100 ml.) is undoubtedly abnormal, and any between one and one and a half divisions (22 to 30 mgm. per 100 ml.) is suspicious. This view is now accepted by the French Trypanosomiasis Service. In the past, however, there have been wide variations in the limits considered normal by different workers, even when the same methods are used. These variations are shown in the table :—

TOTAL PROTEIN IN NORMAL CEREBROSPINAL FLUID

Method.	Total protein in mgm. per 100 ml. in normal cerebrospinal fluid.	Authors.
Turbidimetric (Diaphanometric)	13–20	Mestrezat (1912).
,, (Mestrezat) .	Up to 30	Buzzard and Greenfield (1922).
,, (Proteinometer)	,, ,, 40	Grey (1930).
,, (Mestrezat) .	10–30	Harrison (1939).
,, (Sulphosalicylic)	20–40	King (1946).
,, (Denis and Ager)	15–40	Stitt *et al.* (1948).
,, (Sulphosalicylic)	20–35	Panton and Marrack (1945).
,, (Proteinometer)	20–30 (35)	Hill (1948).
Precipitation (Aufrecht) .	20–35	Hutchison and Hunter (1934).
,, (Nissl) . .	13–47	Levinson (1939).
,, (Aufrecht). .	20–30	Purves-Stewart (1924).
,, (Sicard and Cantaloube) .	Up to 22	Saunders (1948).
,, (Sicard and Cantaloube).	,, ,, 22	French Trypanosomiasis (A.O.F.) Service.

A comparison made by Hill (1948) shows that the proteinometer consistently gives protein values 1·5 to 2 times greater than the precipitation method of Sicard and Cantaloube. Such a discrepancy would be of considerable importance when the proteinometer indicates from 30 to 40 mgm. per 100 ml. for, according to Sicard and Cantaloube (1916), this figure is grossly abnormal and, according to the view of Fairbairn (1934) in East Africa and most workers on trypanosomiasis in West Africa, it indicates a bad prognosis in sleeping sickness. On the other hand, according to Grey (1930) and others, such a figure is within normal limits, or perhaps, at the worst, only at the upper limit of the normal. It would seem that until a more rapid and more precise method of estimating total protein is available the Sicard and Cantaloube technique is preferable, since it produces a precipitate and thus lends itself to accurate measurement more readily than do the opacity methods. The International Trypanosomiasis Conference at Brazzaville, held in February, 1948, recommended that the upper limit of the normal total protein content of the cerebrospinal fluid should be taken as 25 mgm. per ml., as given by the Sicard and Cantaloube technique. Further investigations are required on the composition of the cerebrospinal fluid in the normal African.

Whereas in the majority of cases successful treatment is associated with a fall in the total protein in the cerebrospinal fluid, there is a small number of cases where the protein content after a primary fall remains at about 30 mgm. per 100 ml. for a considerable period and the patient, though not cured, shows considerable clinical improvement. Sicé (1937) emphasises the "protein plateau" after a primary fall. On the other hand, the protein curve may fail to show any decided fall but may continue to oscillate about a mean. If there is a sudden increase in the total protein content of the cerebrospinal fluid it may be taken as evidence of a relapse, even in the absence of trypanosomes.

Whereas the number of cells may not increase in a relapse, the protein content may show a sudden increase. Very occasionally, as Nattan-Larrier and Ringenbach (1912) pointed out, a patient with marked signs of cerebral involvement may show no increase in protein content in the cerebrospinal fluid.

A blood relapse may occasionally occur while the protein content of the cerebrospinal fluid remains normal or very nearly normal. On the other hand, certain apparently cured cases continue to show a protein level of just over 30 mgm. per 100 ml. for years.

It should be noted that the normal protein content of the cerebrospinal fluid is higher than that of the sub-occipital fluid and still more so than that of the ventricles (Barlovatz, 1933 ; Guillain and Mollaret, 1935).

Blood Sedimentation Rate. Although of much less importance than a study of the cerebrospinal fluid, investigations of the blood sedimentation rate by Hollins and Lewis-Faning (1947) showed that the majority of normal healthy Africans have rates of between 10 mm. and 15 mm. per hour, whereas Europeans have rates of less than 5 mm. in one hour. This may be associated with a difference in the albumin : globulin ratio. In trypanosomiasis the sedimentation rates are still further increased and are usually above 20 mm. in the first hour. In trypanosomiasis a low sedimentation rate indicates either an early infection or a long-standing infection of low virulence : a high sedimentation rate means a poor prognosis, since it indicates a relatively low resistance on the part of the patient. Trypanocidal therapy rapidly reduces the high sedimentation rate in trypanosomiasis ; hence determination of sedimentation rates is a valuable auxiliary measure in assessing treatment and in the diagnosis of cryptic infections. During mass surveys sedimentation rates should be determined in all individuals in whom trypanosomes cannot be found. Those persons who have a high sedimentation rate should then be given a single injection of suramin or pentamidine and re-examined a month later. Those who show a pronounced fall in sedimentation rate should be regarded as suffering from trypanosomiasis.

Mass Treatment

Shortly after the first World War the French began an intensive campaign against trypanosomiasis in the French West African Colonies. Diagnostic teams were followed up by treatment teams. Similar schemes were shortly afterwards initiated in the Belgian Congo and in the British West African Colonies. The first survey team in Nigeria was organised in 1930.

The following figures show the work of such survey teams in Nigeria (McLetchie, 1948) :—

Years.	Number of persons examined.	Number of cases.	Average infection rate (per cent.).
1930–35 . . .	1,246,039	169,440	13·6
1936–40 . . .	1,510,304	130,560	8·6
1941–46 . . .	445,952	6,946	1·5

In addition, in Nigeria, the Gold Coast, and Sierra Leone, the Sleeping Sickness Services have set up special dispensaries in areas which have already been surveyed and treated. These dispensaries help in following up cases already treated, in treating the disease in remote areas too far from Government hospitals and dispensaries for rural patients to attend, and in detecting foci where there is a sudden rise in the incidence of the disease. In Nigeria the first sleeping sickness dispensary was established in 1934, and by 1945 there were forty dispensaries functioning; in addition, trained staff of the Sleeping Sickness Service were posted to nineteen native administration dispensaries. By the end of 1946 a total of 499,330 cases had been treated in Nigeria by teams, dispensaries, and hospitals. Some criticism has been directed against the chemotherapeutic activities of the dispensaries. Early cases which have few or no signs or symptoms beyond enlarged cervical lymph nodes do not usually attend dispensaries, since the African does not seek treatment unless he feels ill. Such early cases can be found and treated only by mass survey teams. Mild early cases tend to cease treatment after a few injections unless controlled by a medical officer. In some areas a system has been introduced whereby a chief or leading man goes surety for the attendance of patients from his village. At dispensaries advanced cases do not necessarily get either a thorough examination or the individual treatment which could be given by a medical officer. Thus the rate of cure is considerably lower than when treatment is fully supervised by medical officers. In French territories the Sleeping Sickness Service ensures the treatment of nearly all cases in hospital: the subsequent follow-up is thus considerably simplified.

Mass surveys and treatment also have certain disadvantages. They necessarily entail the maintenance, for an indefinite time, of a sleeping sickness organisation consisting of a highly paid specialised staff for the diagnosis and treatment of this one disease: they do not entirely eradicate the disease unless a very considerable degree of regimentation of the population is accepted, and they do not tackle the question of trypanosomiasis in cattle.

Mass diagnosis and treatment, without other measures of control, are capable of reducing an epidemic to a reasonable level so that hospitals and dispensaries become capable of dealing with it. With an infection rate of 1 per 100 or less any sudden increase in incidence is likely to be quickly discovered and dealt with. Mass treatment, except in special circumstances, cannot eradicate trypanosomiasis, as is shown by the figures in the table, and in some cases the rate may rise. In the Gueckedou district of French Guinea, for instance, in 1946, 119,410 persons out of a total population of 175,547 were examined and 1,202 new patients were detected and treated, but in 1947 the incidence of sleeping sickness was 8·0 per cent. of the population.

INFECTION RATES (PERCENTAGES) SHOWN BY MASS SURVEYS

	Before mass treatment (percentage).	After mass treatment (percentage).	Reference.
Sierra Leone:			
Liberian frontier	5·6 (1941–42)	1·3 (1948)	Davey (1948)
General	2·2 (1941–42)	0·5 (1948)	
Gambia (North Bank)	6·9 (1939)	0·9	Ann. Rept. (1943).
Nigeria (part)	8·9	0·9	Col. Dev. Fund (1940).
,, (mines)	45·4 (1934)	1·41 (1938)	Ann. Rept. (1939).
,, (part)	14·3 (1934)	3·7 (1936)	Ann. Rept. (1939).
,, (whole)	20·5 (1935)	2·1 (1943)	Lester (1945).
French Cameroons	35–45 (1926–28)	5 or less (1934)	Ledentu (1934).
Yaoundé	36–52 (1926)	0·9–1·3 (1930)	Jamot (1932).
,,	17·8	0·04 (1930)	
Akonolinga	42 (1922–23)	2 (1930)	
Lomié	60 (1926)	4·1 (1930)	
Bertoua	28·6 (1928)	0·2 (1930)	
French West Africa	7·4 (1910)	0·2 (1931)	Brazzaville (1932).
French West Africa and Togo	2·11	0·74 (1942)	Muraz (1943).
Ivory Coast	2·0 (1939)	0·25 (1946)	Friedheim (1948).
French Guinea (Gueckedou District)	1·0 (1946)	8·0 (1947)	Friedheim (1948).
Belgian Congo	1·0 (1928)	0·27 (1938)	van Hoof (1938).
Lake Albert	10·0 (1923)	0 (1927)	Davey (1948).

In some small areas it appears to be possible to eradicate the disease almost completely by means of mass diagnosis and treatment. Thus in the Belgian Congo, near Lake Albert, it is claimed that human trypanosomiasis has been entirely eradicated from a population of 100,000, which in 1923 had an infection rate of 10 per cent. The last case of sleeping sickness occurred in 1927, though flies are as numerous as ever and the population is unchanged Similarly, in the French Cameroons trypanosomiasis has been, it is claimed, completely eradicated solely by mass diagnosis and treatment. The diagnostic methods have been especially thorough, the blood of every person being examined at every survey, as well as lymph node juice and, when necessary, cerebrospinal fluid. A reduced Sleeping Sickness Service is now maintained in the French Cameroons for observation purposes and to prevent imported cases from starting a fresh epidemic.

The majority of observers in other areas have failed to eradicate infection by treatment alone (Ledentu, 1934 ; MacQueen, 1938 ; Lester, 1939 ; van Hoof, 1940 ; Muraz, 1943 ; Vaucel, 1941 ; Diaz Varela, 1944).

These failures are due in most instances to a variety of causes. In some areas, such as the Benue Province of Nigeria, only 56 per cent. of patients completed their full course of treatment. Some undoubtedly were cured even by incomplete courses, but trypanosomes after any blood relapse are still infective to tsetses. It is by no means easy to re-examine all patients treated some time previously, more especially as in many areas a considerable part of the male population migrates from time to time in search of work. While some patients may be away from home at the time of the visit of the treatment team, others may be deliberately hidden because of shame, fear or for religious reasons. Other members of the community may avoid examination because of their social position. *T. gambiense* infections are not always easily diagnosed, for the numbers of parasites appearing in a preparation of blood or lymph node juice are often very small. Under the difficult conditions of field diagnosis some cases are inevitably missed. So long as the population is in contact with *Glossina* there exists the danger of the reintroduction of infection by travellers from other zones and by relapse-cases showing trypanosomes in their blood. In addition, mass treatment leaves untouched the supply of infected tsetse flies. Whereas man is probably the only host for *T. gambiense*, there is more than a suggestion that alternative hosts exist for *T. rhodesiense*. When it is considered that a mass-treatment campaign involves the training and employment of several hundred African technicians who have to work under difficult bush conditions with highly superstitious and often very primitive people, it would not be surprising if the resultant leakage of infections past the teams is considerably greater than is usually suspected. Vaucel (1941) notes that whereas considerable success may be achieved in the spreading zones of an epidemic, failure is not uncommon in old endemic foci situated in swampy riverine areas with large numbers of tsetses. Here chemotherapy alone allows only very partial success. Apart altogether from resistant strains, old foci may flare up again after having been quiescent for some years. The stage of the disease at which treatment is begun may also have an important bearing on the results of mass treatment. If a high proportion of cases is already in the second stage the percentage of success will be less than when the majority is recently infected. Thus in the Old Kudaru district of Nigeria, where most cases were in an early stage, an examination of 945 out of the 1,575 cases treated three years previously showed 3 positive on lymph node puncture.

There is general agreement that resistance to arsenical drugs is more frequent and more widespread than it was twenty years ago. Whether this is due to the development of arsenic resistance in patients insufficiently treated, or whether, as suggested by van Hoof *et al.* (1938), and van Hoof (1947), it is due to the fact that among the natural trypanosome population there are degrees of arsenic resistance and all the more susceptible trypanosomes have now been eliminated, is not yet certain. The areas in which drug resistance is known to exist in West and Central Africa are discussed on p. 494.

Thus, although in a few areas where the patients are all in the early stage chemotherapy alone may succeed in eradicating the infection, the general consensus of opinion is that in most parts of West and Central Africa chemotherapy must be combined with

" aggressive " bush-clearing, for only in this way can the contact between man and the tsetse fly be reduced to a minimum.

Morris (1946) has compared the effects on the incidence of trypanosomiasis of aggressive clearing as carried out in the Northern Territories of the Gold Coast on the one hand with the results from mass diagnosis and treatment as carried out in the adjacent French Ivory Coast. In both areas anti-sleeping sickness measures were begun in 1939 : in French territory with biannual examinations and treatment of the population by mobile teams and bush-clearing only at obvious points of man-tsetse contact such as ferries and main road crossings over rivers. In the Gold Coast, complete eradicative clearing was combined with intermittent mass treatments in certain selected districts only. Whereas in the Gold Coast the incidence of sleeping sickness in all areas of eradicative clearing has gone down, in French territory there have been some areas where the incidence has remained stationary or has increased.

The weaknesses inherent in mass treatment as the only measure might theoretically be decreased by increasing the frequency of visits, but this would add greatly to the cost, already high, and might raise difficulties such as the resistance of the people to too frequent interference. A more logical measure is improved entomological control.

The need for effective tsetse control can be seen, as pointed out by Morris (1946), from an analysis of the potentials for the propagation of the disease that remain after different methods of control have been applied :—

$$\text{(1) Mass-treatment leaves—}\left\{\begin{array}{l}\text{Cases missed + re-}\\\text{duced number of in-}\\\text{fected flies}\end{array}\right\}\left\{\times\ \begin{array}{l}\text{full vector}\\\text{potential.}\end{array}\right.$$

$$\text{(2) Tsetse reduction leaves—}\left\{\begin{array}{l}\text{Infected population}\\\text{+ reduced number}\\\text{of infected flies}\end{array}\right\}\left\{\times\ \begin{array}{l}\text{reduced}\\\text{vector}\\\text{potential.}\end{array}\right.$$

(3) Tsetse eradication leaves—Infected population only.

$$\text{(1) and (2) leave—}\left\{\begin{array}{l}\text{Cases missed + re-}\\\text{duced number of in-}\\\text{fected flies}\end{array}\right\}\left\{\times\ \begin{array}{l}\text{reduced}\\\text{vector}\\\text{potential.}\end{array}\right.$$

(1) and (3) leave—Missed cases only.

Thus, if tsetses can be eradicated, mass treatment merely increases the speed of control and has no effect on the end result.

The development of chemoprophylaxis with pentamidine has undoubtedly increased the usefulness of drugs in the control of trypanosomiasis in man. However, certain of the difficulties met with in mass treatment are still present in mass chemoprophylaxis. Some persons will fail to have a prophylactic injection, others who have received an injection will be in the early stages of infection. If tsetses still have an opportunity of biting infected persons the disease will not be eradicated since some time after the prophylactic injection a number of susceptible persons will still be available. Trypanosomes may be exposed to sub-lethal doses of a drug present in the blood stream sometimes after a prophylactic injection: drug resistance may thus be encouraged. As Vaucel (1941) has emphasised, purely medical work can be completed only by the agronomic, economic, and social transformation of the country. The truth of this statement is amply borne out by the account given by Nash (1948) of the Anchau Development and Resettlement Scheme.

Trypanosoma rhodesiense Infections

The treatment of infections due to *T. rhodesiense* has always been recognised to be less easy than that of infections caused by *T. gambiense*, and some workers believe that if treatment is delayed more than six weeks after the onset of symptoms the chances of permanent cure are small.

Suramin was for a time given alone to patients with early infections. Maclean (1929), for instance, gave 4 gm. of suramin in three or four doses spread over a month. To allow a margin of safety, however, 8 gm. was usually administered and this was followed by tryparsamide in doses of 2 to 3 gm. at weekly intervals until at least 36 gm. had been given. Maclean and Fairbairn (1932) also at first gave suramin alone to early cases, but where the prognosis was in any doubt combined treatment was found to be preferable. Keevil (1934) gave the subsequent history of six patients who originally showed *T. rhodesiense* in the cerebrospinal fluid and had been treated with suramin only: three were alive and well eight years later, while three had died after two and a quarter, five and eight years: the cause of death was not sleeping sickness in one and was unknown in the others.

Of the 719 patients treated by Maclean and Fairbairn (1932) in the Maswa district of Tanganyika, where patients came comparatively late for treatment, only twenty-six of 131 (16 per cent.) were completely cured after an observation period of more than six and a half years. In the Tabora (epidemic) area, where patients came for treatment relatively early, of 588 patients treated, 283 (48·2 per cent.) were well and probably cured after an observation period of not less than two years and five months.

Coghlan (1933) gave 5 gm. of suramin in weekly doses of 1 gm. followed after a month's rest by at least 20 gm. of tryparsamide, 3 gm. being the weekly dose. Fairbairn (1944) has recorded the present methods of treatment. If the proteins in the cerebrospinal fluid is not above 30 mgm. per 100 ml. by the method of Sicard and Cantaloube the patient can be cured by suramin: if the protein is between 30 and 35 mgm. per cent. a full course of tryparsamide must follow the suramin: if protein is above 40 mgm. per cent. the case is incurable. A reasoned prognosis can be given only if the protein in the cerebrospinal fluid has been estimated at the beginning of treatment and during an adequate follow-up period. This is of course true of all forms of sleeping sickness in Africa (Fairbairn, 1934).

In any outbreak of sleeping sickness in a new area many patients are almost certain to be at an advanced stage. It takes some months before the results of treatment and of propaganda make patients come at an early stage for treatment. The African, however, as Saunders (1944) has pointed out, is perfectly capable of understanding the reasons for early treatment and for continuing with the course to the end.

The cure rate of 48·2 per cent. recorded for the Tabora epidemic is, according to Fairbairn (1948), about the best that can be expected in a large epidemic due to *T. rhodesiense*. Of the 23,955 patients diagnosed between 1922 and 1946 in Tanganyika at least 11,500 have died or will die from sleeping sickness. This is a serious loss to a country which is already under-populated. It is obvious that treatment alone is not the answer to the problem of how to treat trypanosomiasis in East Africa due to *T. rhodesiense*. It would seem essential to reduce contact between man and fly.

One method of thus reducing contact is to ensure that all relapse cases are given 1 gm. of suramin monthly so that the blood is no longer infective (Fairbairn, 1944).

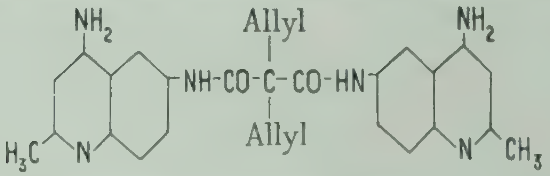
In East Africa, owing to shifts in population, elimination of wild game animals and bush-clearing, human trypanosomiasis has been reduced to manageable proportion (Fairbairn 1943). At present there appear to be about 2,000 new cases per annum with approximately 300 deaths a year in East Africa, with the exception of the Anglo-Egyptian Sudan (Buxton, 1948). There are, however, two complicating factors. Healthy human carriers of *T. rhodesiense* undoubtedly exist both in Southern Rhodesia (Blair, 1939) and Nyasaland (Lamborn and Howat, 1936), and Fairbairn and Burtt (1946) have found that in volunteers the Tinde strain produces very mild infections. The second factor is that *T. rhodesiense* tends to produce epidemics of considerable magnitude with extreme rapidity, as in Eastern Uganda in 1940 (Mackichan, 1944).

So far little has been done to correlate mass diagnosis and treatment with cycles in the tsetse population. Studies in Tanganyika by the Department of Tsetse Research have shown that routine catches of *Glossina swynnertoni* in unaltered blocks of bush have fluctuated apparently with a long-term rhythm since at any rate 1930, with highest catches at sunspot minima (1934, 1944), and lowest catches at sunspot maxima (1937, 1947). These figures for *G. swynnertoni* further exhibit some agreement with figures for *G. pallipedes* both in Tanganyika and Zululand, and with numbers of cases of human trypanosomiasis (Fairbairn, 1948). In the course of this long-term fluctuation the natural reduction of *G. swynnertoni* may be as much as 90 per cent. from peak to trough (Jackson, 1949). Intensive mass treatment and prophylaxis with drugs should therefore be most effective when tsetses are lowest in numbers. Although in many areas in East and Central Africa human trypanosomiasis is being brought under control, trypanosomiasis of domestic animals still remains a most important problem in both East and West Africa.

The Treatment of Infections due to *T. cruzi*

The reaction of *T. cruzi*, the causal agent of Chagas's disease, to chemotherapeutic drugs differs very considerably from that exhibited by other pathogenic trypanosomes. Many drugs which are highly active against old-world trypanosomes have little or no action on *T. cruzi*.

Earle (1946) believed that penicillin was responsible for the cure of one case, but others (Neghme, 1945 ; Talice, 1945) have entirely failed to show that penicillin is of value. The older arsenicals are without action and so is butarsen (Talice and López-Fernández, 1945) : N-*p*-*iso*propylbenzylethylene diamine was shown by Lwoff *et al.* (1944) to have some action against *T. cruzi* in mice. More interest attaches to a quinoline derivative, Bayer 7602 (Ac). This compound, which is derived from the surface antiseptic surfen A, contains two 4-amino-2-methylquinoline groups joined by a diallyl malonyl group in position 6 to form diallylmalondi [4-amino-2-methyl-quinolyl-6-amide] (Bios, 1947).



It was originally prepared by Iensch (1937).

In experimental animals (Mazza, 1941 ; Fulton, 1943) the drug appears to exercise no influence on the motility of the flagellates or on their morphology and, according to Mazza (1941), it has no action on the leishmania-like forms in the tissues. Fulton (1943) found that the toxicity for mice in mgm. per 20 gm. mouse varied as follows, according to the route of administration :—

Oral	>96
Subcutaneous	>96
Intraperitoneal	3
Intravenous	0·1

Subcutaneous administration causes severe ulceration. Parasites disappear rapidly from the blood stream and, regarding this as evidence of cure, Mazza (1941) believed that the chemotherapeutic index for blood forms was 1 : 50. Doubt has been thrown on this finding by Fulton (1943), who shows that though parasites disappear from the blood stream they may reappear for the first time some eighty-nine days later.

Bayer 7602 (Ac) was first supplied in a 3 per cent. solution, but later it was dispensed in ampoules as a solid which, when dissolved in water, causes much less pain.

Mazza, Cossio and Zuccardi (1937) first used this compound in the treatment of a three-months-old child to whom a dosage of 18 mgm. per kilo. body weight was given intramuscularly. Trypanosomes disappeared from the blood, but xenodiagnosis showed that sterilisation had not been effected. According to Mazza, Freire and Salica (1942), treatment of the meningo-encephalitic form of Chagas's disease effected cures when the drug was given intramuscularly in doses of 100 mgm. per kgm. Death, however, is known to have occurred in eleven out of twenty-nine cases.

Mazza (1941), as a result of treating and studying 122 cases in the early stages of the disease, considered that adequate dosage in man ranged from 30 to 120 mgm. per kgm., according to the age and physical condition of the patient. In further communications Mazza *et al.* (1942 a, b) reviewed their results after five years use of the drug. During the war years the German product was unavailable in South America and a similar product was therefore produced in Great Britain: its toxicity and therapeutic effects were similar to those of the German product. Mazza *et al.* (1945) claim to have used the British product (M.3024) with considerable success. The initial dose is 7 to 12 mgm. per kgm. of body weight and the drug is given at intervals of five to seven days, the second dose being twice the first. Acute symptoms, such as œdema of the face and eyelids, disappear rapidly, but further studies are required to determine whether permanent cures have been obtained.

The drug has little value as a prophylactic (Fulton, 1943). It is true that the incubation period may be prolonged by small as well as by large doses, but complete protection was not afforded by any dose of drug when inoculation with *T. cruzi* was carried out two weeks later, although in general the period of latent infection corresponds in length with the size of the dose and with the interval between drug treatment and inoculation. Subcutaneous inoculation caused extensive ulceration and intravenous injection was valueless, possibly because the drug is quickly excreted by this route.

Mazza *et al.* (1942a) also tested another compound, Bayer 9736 (Ac), of undisclosed composition, which acts on a number of other trypanosomes as well as on *T. cruzi*. The new preparation contains arsenic and sulphur and is less toxic than 7602 (Ac), but also less active against *T. cruzi*. Browning, Calver, Leckie and Walls (1946) report that 3-carbethoxyamino-9(*p*-carbethoxyaminophenyl)-10-methyl phenanthridinium chloride (No. 1544) is active against *T. cruzi* in mice in a narrow range of doses : it has no action on *T. brucei* or *T. congolense*.

Up to the present therefore no satisfactory chemotherapeutic drug has been found for the treatment of Chagas's disease. The presence of tissue forms which are doubtless responsible for the reappearance of parasites in the peripheral blood after treatment is a complication which has yet to be overcome, for the tissue forms are far less easily destroyed than those in the peripheral blood stream.

The Treatment of Trypanosomiasis in Domestic Animals

The treatment of trypanosomiasis in cattle is still an immense problem of paramount importance to the future of Africa. It has already been discussed in relation to the use of phenanthridinium compounds and antrycide, which are both far more effective than older drugs in the treatment of infections due to *T. congolense* and *T. vivax*. The rapid development of resistance to antrycide is, however, a serious drawback. In camels some results are still claimed for the use of tartar emetic, but suramin appears to be more effective (Grassi, 1947). In surra, the disease of horses due to *T. evansi*, a combination of suramin and atoxyl is of little value but, according to Yutuc (1941), suramin and sodium antimonyl tartrate successfully cured two of five experimentally infected and one of three naturally infected horses. Surra is said to be much more difficult to cure in the later stages of infection (Yutuc, 1934). Phenanthridinium compounds have so far proved unsatisfactory in horses. Quindoline methochloride was found by Calver (1945) to be curative in mice in doses approaching the maximum tolerated. Severe local reactions were produced : the hydrochloride was inert. Penicillin also is without action on *T. congolense* (Bessemans *et al.*, 1944). Apart from a few cases cured by antrycide, no drug has any constant action on *T. simiæ* which produces devastating epidemics in pigs.

The use of a chemoprophylactic drug against *T. congolense* and *T. vivax* opens up fresh possibilities, but apart from the danger of producing drug-fast strains most of the difficulties encountered in effective immunisation of men are not only met with but are intensified in the case of cattle. To persuade the nomadic Fulani, a people highly suspicious of all European interference, that all their cattle must be injected at least every six months will be by no means a simple task.

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THE CHEMOTHERAPEUTIC ACTION OF DRUGS ON TRYPANOSOMIASIS

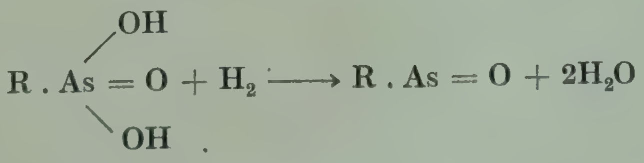
Despite the intensive study of trypanosome infections and their reactions to drugs, many problems still require considerable investigation before any true picture is obtainable of the means by which trypanosomes are destroyed by drugs. Thus it is still uncertain how far eradication of trypanosomes from the body is due to drug action alone, to the combined action of drugs and reproduction-inhibiting bodies (ablastin), or to the combined action of drugs, ablastin, and specific immune bodies.

In considering the means by which trypanosomes are destroyed in the body by drugs a number of phenomena require consideration :—

(1) The development of trypanocidal activity *in vitro* and *in vivo.*
(2) The rate of absorption and excretion of trypanocidal substances by the tissues.
(3) The action of trypanocidal drugs on trypanosomes.
(4) The specific relationship between arsenical compounds and certain trypanosome infections.
(5) Synergistic action.
(6) Spontaneous changes in the reaction of strains of trypanosomes to drugs.
(7) Immunity and chemotherapy.
(8) The *rôle* of the reticulo-endothelial system.

(1) The Development of Trypanocidal Activity *in vitro* and *in vivo*

Since the arsonic acids are relatively inactive in the destruction of trypanosomes *in vitro* but active *in vivo* whereas the arsenoxides are active *in vitro,* it was thought by Ehrlich (1909a, b) that the tissues must have the power of reducing the quinquevalent arsonic acids to the tervalent arsenoxides which then destroy the trypanosomes :—



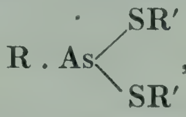
$$\mathrm{R\,.\,}\overset{\diagup\mathrm{OH}}{\underset{\diagdown\mathrm{OH}}{\mathrm{As}}}=\mathrm{O}+\mathrm{H_2}\longrightarrow\mathrm{R\,.\,As}=\mathrm{O}+2\mathrm{H_2O}$$

Arsenical compounds were thus divisible into the quinquevalent arsonic acids on the one hand and the tervalent compounds, arsenoxides and arseno-compounds, on the other.

This theory, now generally accepted, was extended by Voegtlin and Smith (1920a, b), and by Voegtlin, Dyer and Leonard (1923), who showed that *in vivo* the time required for quinquevalent arsenic and antimony derivatives to exert trypanocidal activity is longer than for tervalent compounds. This longer latent period, it was assumed, must be due to the gradual reduction of a sufficient amount of the drug to the tervalent form. Since too arsenic in the arsenoxide form acts much more rapidly than arsenic in the form of an arsphenamine derivative it is suggested that these latter compounds are oxidised in the body to the oxide :—

$$R . As = As . R + O_2 \longrightarrow 2R . As = O.$$

This conception is strengthened by the fact that the toxicity of a slightly alkaline solution of either arsphenamine or neoarsphenamine is increased when kept in contact with air, probably owing to oxidation to *meta*-amino-*para*-hydroxyphenyl-arsenoxide, since on treating arsphenamine in alkaline solution with hydrogen peroxide, Ehrlich and Bertheim (1912) produced *meta*-amino-*para*-hydroxyphenylarsonic acid. In addition, an aqueous solution of the sodium salt of arsphenamine which has been incubated for three hours at 37° C. exerts a much greater trypanocidal activity than freshly prepared solutions and has no latent period in its trypanocidal action. Since reduction of the quinquevalent arsenicals to the arsenious oxide stage would require a longer time than oxidation of the arsphenamines to the same stage, a longer latent period for trypanocidal action would be necessary for the arsonic acids than for the arsphenamines. This has been found to be the case by Voegtlin and Smith (1920a and b), the latent period for the arsphenamines being from one to three hours, for the quinquevalent arsonic acids from twelve to eighteen hours.

The difference in action of quinquevalent and tervalent arsenicals was reinvestigated by Yorke and Murgatroyd (1930), using the method elaborated by Yorke, Adams and Murgatroyd (1929) for maintaining pathogenic trypanosomes alive and active *in vitro* at 37° C. for at least twenty-four hours. Whereas the quinquevalent compounds had very little trypanocidal action *in vitro*, all compounds containing tervalent arsenic, whether arsenoxides of the type $R . As = O$, thioarsinites  $R . As\begin{matrix} SR' \\ SR' \end{matrix}$, or arseno-compounds $R . As = As . R$, were lethal to trypanosomes in high dilution. Like Ehrlich, Yorke and his colleagues thus divided arsenical compounds into two groups, the weakly toxic arsonic acids and the toxic tervalent compounds, the therapeutic action of which was ascribed to the direct trypanocidal action of the unchanged drugs. So far as quinquevalent arsenicals are concerned, it is generally agreed that their toxicity is small not only to trypanosomes but to spermatozoa and to cells in tissue culture (Levaditi and Constantinesco, 1932); whereas, as Yorke and Murgatroyd (1930) have shown, the minimal lethal concentration of quinquevalent arsenicals *in vitro* for *T. rhodesiense* is 1 in 1,600, reduction to the tervalent form increases the minimal lethal concentration to 1 in 200,000,000.

Fresh light has been thrown on the action of arsenoxides by a reconsideration of their trypanocidal activity by Eagle (1945) who investigated the trypanocidal and spirochæticidal action of acid-substituted phenyl arsenoxides as a function of pH and dissociation constants.

With very few exceptions the acidic groups cause a considerable decrease in the activity of the parent phenyl arsenoxide, the inhibition not being referable to an effect on the dissociation of the $—AsO$ or $—As(OH)_2$ group, since the unsubstituted phenylarsenoxide gives the same titration curve in the appropriate pH range (pH 5·5 to 9·0) as its acid-substituted derivatives (Eagle *et al.* 1940). Diminished activity is rather to be explained on the view that the ionised salts of these substituted compounds are relatively inactive as compared with the undissociated free acids. This is in accordance with the following facts: (1) the dissociation constants of most of these compounds are such that at pH 7·4 they are more than 99 per cent. ionised, and (2) the increase in the hydrogen ion concentration of the medium in which the tests are carried out results in increased trypanocidal and spirochæticidal action. Thus the most obvious effect of increasing the hydrogen ion concentration consists in the formation of undissociated free acid from the ionised salt.

$$\mathrm{OAs\,.\,C_6H_4\,.\,COO^- + H^+ \longrightarrow OAs\,.\,C_4H_4\,.\,COOH.}$$

If the inference is correct that the undissociated acid is many times more active than the charged ion there should be a close correlation between the trypanocidal activity of an acid-substituted compound at a given pH and the pK of that compound. Theoretically the higher the pK, or the weaker the acidic group, and thus the higher the proportion of the undissociated acid at a given pH, the greater must be the activity of the compound. This was found to be the case experimentally.

Confirmation that the influence of pH on the trypanocidal action on *T. equiperdum* of acid-substituted phenyl arsenoxides is related to the ionisation of the acidic group is borne out by a number of other findings. The activity of the unsubstituted phenyl arsenoxide is largely independent of the pH of the solution in which it acts: this is true also of 3—NH_2—4OH phenylarsenoxide in which neither of the substituents is strongly acidic. Even more significant is the fact that when an acidic group is blocked as by amide formation, as in the p-$CONH_2$ compound, trypanocidal activity is then independent of pH changes.

The ionised salts of acid-substituted phenyl arsenoxides have in general a relatively low, though variable, degree of trypanocidal activity, but in a few exceptional cases, as in the p-$(CH_2)_3COOH$ compound, the ion is unusually active. This, when combined with the activity due to the undissociated molecule, accounts for the high trypanocidal potency of these compounds.

It is considered that there is sufficient correspondence between theoretical calculations and observed facts to conclude that the relative trypanocidal activity of an acid-substituted phenyl arsenoxide is determined by and is roughly predictable from: (*a*) the pH of the solution; (*b*) the pK of the compound, and (*c*) the trypanocidal activity of the ion. The higher the pK, and the lower the pH, the greater will be the trypanocidal activity of the compound.

Arsenic estimations show that the activity of these compounds can be correlated with the amount of arsenic bound by the trypanosomes. Salts of the compounds are bound to the trypanosomes only to a minor degree, whereas the undissociated free acids are concentrated sometimes as much as three-hundred fold. Variation in pH of the solution, since it affects the relative proportions of ions and undissociated acid, similarly affects the degree to which a given arsenical is bound by the trypanosomes. In a series of acid-substituted phenyl arsenoxides those with the highest pK are the first to show increased activity and increased affinity for the trypanosomes as the pH of the reacting mixture is decreased by acid; those with strongly acidic groups which remain as ions at the lowest pH tested are unaffected with respect both to trypanocidal activity and affinity for combining with trypanosomes.

The question arises whether the undissociated acid-substituted phenyl arsenoxides are so strongly bound to trypanosomes because they have an especial affinity for cellular constituents, such as those containing —SH groups. It is probable, however, that the undissociated acids penetrate readily into the interior of the trypanosomes whereas the ionised salts are unable to do so. It seems, in fact, to be a general rule in biology that the ions of weak acids or bases pass through cell membranes less readily than the corresponding undissociated molecules. In addition, the activity of weak bases is increased by alkalinisation rather than acidification (the toxicity of quinine for *Colpidium* (Prowazek, 1910) or *Paramecium caudatum* (Crane, 1921)). The effect of quinine (dissociation constant $2{\cdot}2 \times 10^{-7}$) on the isolated frog heart also increases with alkalinisation in the pH range 6·5–8·0, due, according to Goljachowski (1934), to the formation of the undissociated free base which is bound by the heart tissue.

A distinguishing feature of the trypanosome-arsenoxide system is the speed, and particularly the degree, to which active non-ionised arsenicals are concentrated by the trypanosomes. Within ten minutes the arsenic concentration in the organisms may attain a level several hundred times greater than that in the surrounding fluid. There must therefore be a rapid diffusion of the arsenic into the organisms followed by its firm combination with cellular elements. The arsenical is thereby effectively removed from the diffusion equilibrium, permitting its continued diffusion into the cell. In comparison with the effect of this combination in permitting the accumulation of arsenic in the trypanosomes, the partial conversion of some of the acid to the ionised form within the cell is a quantitatively negligible factor.

Since quinquevalent arsenic is only feebly trypanocidal *in vitro* but becomes active *in vivo*, the question arises how this activation is brought about. The work of Levaditi and Yamanouchi (1908), and of Levaditi (1909a, b) showed that when atoxyl was incubated at 37° C. with an emulsion of liver, kidney, brain or muscle, trypanocidal properties arose in the mixture due to the formation of a hypothetical substance " trypanotoxyl." Durel and Ratner (1944) reported their inability to confirm this early work. Extracts of fresh liver kept at room temperature or at 37° C. did not activate atoxyl but possessed trypanocidal properties on their own account. Levaditi and Vaisman (1946) therefore decided to re-examine the whole question. It was confirmed that fresh liver extract from rabbits is harmless to *T. equiperdum* : it does, however, render atoxyl trypanocidal *in vitro*. This property is retained for fourteen days at − 20° C. and for two to four days at + 4° C. In the absence of atoxyl such tissue preparations exhibit no trypanocidal properties, certainly for fourteen and seven days respectively. However, when livers are kept at room temperatures (20° C.) they acquire trypanocidal properties within two days as autolysis develops, the potency of this trypanocidal function increasing up to seven days, the limit of observation. The trypanocidal activity thus arising supplements that due to activation of atoxyl, when extracts prepared from such livers, after keeping at 20° C. for periods up to four days, are brought into contact with the drug. Liver autolysis proceeds either under sterile conditions or in the presence of bacteria, particularly *Pasteurella*. These organisms as well as *Bacterium coli* and staphylococci, are capable of lysing trypanosomes *in vitro* at 37° C. Thus the results reported by Durel and Ratner may have been due either to the fact that autolysis had already begun in their liver preparations or to the presence of bacteria.

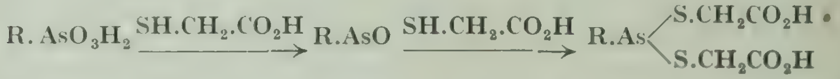
The trypanocidal factors in autolysed liver are rather more thermolabile than the atoxyl-activating components of fresh liver: the former are destroyed, the latter only slightly decreased, by heating at 100° C. for thirty minutes.

Yamanouchi (1910) concluded that the trypanocidal substance was produced by red blood corpuscles because liver and other organs freed from blood no longer possess the power of activating atoxyl. Red cells act more powerfully in the presence of an excess of carbon dioxide than under normal conditions and in the presence of oxygen they fail to activate atoxyl: pure recrystallised hæmoglobin is without action. The active substance is soluble in alcohol, thermostable, and free from protein. Terry (1912) found that both liver and blood, when incubated with atoxyl, transformed the drug into a highly toxic substance. The transforming agent in liver had, however, characteristics which in some respects differed from those of the active agent in blood. The transforming agent in blood is thermolabile, but the toxic substance into which atoxyl is transformed is thermostable (Terry 1915). Levaditi, Anderson and Manin (1928) therefore suggested that the constituent in the tissues which converts atoxyl into a trypanocidal substance is reduced glutathione, a view taken later by Singer and Fischl (1935a). However, the relationship between the glutathione content of the organs and their capacity to transform atoxyl into trypanotoxyl is neither absolute nor constant. It is therefore argued that there must be other factors operating in addition to glutathione, since when atoxyl and tissues are incubated together, some glutathione always remains in the tissues and even after extracting tissues with trichloracetic acid to remove glutathione the tissues retain the power of forming trypanotoxyl. Another reducing substance, cysteine, does in fact act like glutathione, but a third, ascorbic acid, does not. Hydrogen peroxide also is inactive in this respect (Levaditi and Vaisman, 1946). Blood which is poor in glutathione is capable of activating atoxyl, thus suggesting that glutathione is not the only compound capable of acting on atoxyl.

The relationship of the red blood cells to activation of quinquevalent arsenicals was studied by Lourie, Murgatroyd and Yorke (1935). Both reduced tryparsamide and tryparsamide diffuse into red cells and that part of the tryparsamide which enters the red cell is there changed into a more actively trypanocidal substance. Although a solution of laked red cells greatly increases the trypanocidal power of tryparsamide, the agent which activates the tryparsamide is neither reduced hæmoglobin, oxyhæmoglobin nor carboxyhæmoglobin. The activating substance is relatively thermostable, resisting almost completely 65° C. for thirty minutes: it is not completely destroyed by heating at 75° C. for thirty minutes. The activating power of the red cell solutions kept at 0° C. is, however, gradually lost so that within two months or less such stored solutions become practically inert.

While the evidence is thus considerable that the quinquevalent arsenicals are reduced in the body to arsenoxides, there is still some uncertainty as to whether the thioarsinites and arsphenamines exert their curative action in the unchanged condition, or after oxidation to the active arsenoxide R . AsO.

Friedberger (1908) foreshadowed the significance of the thioarsinites as chemotherapeutic agents when he found that *p*-aminophenylarsonic acid mixed with thioglycollic acid became more toxic to mice as well as trypanocidally active *in vitro*. Bertheim (quoted by Roehl, 1909) showed by analogy with arsenious acid that this was due to the production of arsenoxide by reduction, with subsequent formation of thioarsinite, as shown in the following :—



$$R.AsO_3H_2 \xrightarrow{SH.CH_2.CO_2H} R.AsO \xrightarrow{SH.CH_2.CO_2H} R.As\left\langle\begin{matrix}S.CH_2CO_2H\\S.CH_2CO_2H\end{matrix}\right.$$

Much later Voegtlin, Dyer and Leonard (1923) prepared di-(carboxymethyl)3-amino-4-hydroxyphenyl thioarsinite by heating together 3-amino-4-hydroxyphenylarsenoxide and thiolactic acid. This compound showed a definite delay in trypanocidal action as compared with the parent arsenoxide. Strangeways (1937) also studied the action of a number of thioarsinites prepared by condensation of aromatic arsenoxides and sulphydryl compounds such as glutathione and cysteine (Cohen, King and Strangeways, 1931a and b, 1932a). All these compounds were less toxic than their arsenoxide content would suggest, and in many cases a substance with a high therapeutic index was produced by condensation with the sulphydryl compound of an oxide having a low therapeutic index. Thioarsinites have a toxicity for trypanosomes *in vitro* in high dilution. This toxicity is due to the hydrolysis of these compounds in aqueous solution with liberation of the highly trypanocidal arsenoxide, as is shown by (1) the identity in the lethal activity of equimolecular concentrations of a thioarsinite and its parent oxide, (2) the inhibition of the lethal action both of an arsenoxide and a thioarsinite in strong solution by the addition of ten molecules excess of glutathione, and the failure to obtain protection in higher dilutions owing to more extensive hydrolysis. The inhibitory action of an excess of glutathione on the therapeutic action of the thioarsinites, due to the inhibition of the formation of arsenoxide, is thus similar to the inhibitory action of glutathione on the chemotherapeutic action of the 3-amino-4-hydroxyphenylarsenoxide. But though the directly trypanocidal action of the thioarsinites is due to arsenoxide, the fact that thioarsinites are more effective in experimental trypanosomiasis than the corresponding arsenoxides is explained by Strangeways (1937) as follows. When thioarsinites, which are readily obtained in solution as neutral sodium salts, are introduced into the blood stream in relatively high concentrations (1 in 1,000 to 1 in 100), there is very little hydrolysis to the arsenoxide and sulphydryl components, as shown by a negligible nitroprusside reaction. After intravenous injection and subsequent dilution there will be some hydrolysis with liberation of free arsenoxide. This partial hydrolysis is rapid, since the trypanosomes begin to disappear from the blood stream within half an hour after injection : but it is not immediately complete since the dose of a thioarsinite which can be tolerated is greater than would be expected from the calculated content of arsenoxide.

Neoarsphenamine has a trypanocidal action *in vitro* in low concentrations, a fact amply verified by the work of Yorke and Murgatroyd (1930), but the lethal concentrations are higher than those found for arsenoxides and thioarsinites. The addition of ten molecules excess of glutathione to strong solutions of neoarsphenamine inhibits its lethal action, though a similar excess of glutathione is without effect on the toxicity of high dilutions. Moncorps and Bohnstedt (1934) found that concentrations of glutathione of 1 in 100 to 1 in 500 reduced the trypanocidal activity of a 1 in 700 dilution of neoarsphenamine both *in vivo* and *in vitro*.

Cysteine has a protective action against neoarsphenamine similar to that of glutathione. This lends support to the suggestion that neoarsphenamine is absorbed by the trypanosomes and becomes oxidised, either within the body of the trypanosomes or on their surface, to the highly trypanocidal arsenoxide. In support of this view are the observations of Gonder (1912), Castelli (1913) and Simić (1923), who showed that both *in vitro* and *in vivo* trypanosomes which have been in contact with neoarsphenamine for from five to thirty minutes retain their motility but no longer infect mice. Papamarku (1927) found that if a mixture of trypanosomes and spirochætes are treated with varying concentrations of neoarsphenamine the trypanosomes are killed after two hours by a dilution of 1 in 10,000,000, but the spirochætes remain healthy for a number of hours in much higher concentrations. Spirochætes, however, were killed by dilutions of 1 in 500,000 after twenty-four hours and 1 in 20,000,000 after forty-eight hours. This difference in time of action on trypanosomes and spirochætes is explainable if trypanosomes are able to oxidise arseno-compounds to the arsenoxide, whereas spirochætes are unable to accomplish this change. The production of arsenoxide by the cells of the host may also play a part in the therapeutic effect of neoarsphenamine. Simić (1923) indeed suggested that this action of the host's cells explains the greater therapeutic efficiency and higher toxicity of neoarsphenamine when given by subcutaneous as compared with intravenous injection. Hasskó (1935) also believes that the cells of the host play a large part in activating neoarsphenamine since, when mice infected with trypanosomes were injected with neoarsphenamine, no neoarsphenamine could be demonstrated within the bodies of the trypanosomes.

So far as non-metallic compounds are concerned, trypanocidal activity appears to be due to the direct action of the unchanged compounds. It was at one time thought that some chemical change in the composition of suramin must occur in the body since its trypanocidal action *in vitro* is very slight whereas *in vivo* it is high. von Jancsó and von Jancsó (1934), however, were able to show that actually the trypanocidal action of suramin *in vitro* is intense provided the drug is allowed to act on the trypanosomes for at least twenty-four hours. By a modification of the method used by Yorke *et al.* (1929), trypanosomes were kept alive and in good condition for from fifty to seventy hours: suramin was then found to be trypanocidal in a dilution of 1 in 80,000 after a latent period of twenty-four hours. Suramin thus differs strikingly from the arsenoxides which have an immediate effect.

The action of suramin on enzyme systems which may be present in trypanosomes is only now receiving the attention which it deserves. In relatively high concentrations such as 0·007 M, or 1 per cent., suramin inhibits trypsin (Beilinsohn, 1929). Town *et al.* (1949) find that trypsin is even more susceptible than was thought. Concentrations of M/1,000 give 30 per cent. inhibition in the hydrolysis of casein and concentrations of M/2,000 give 20 per cent. at pH 8·9 and 30° C. Town and Wormall (1949) suggest that the inhibition of tryptic action may be due to a combination of suramin with either the enzyme, the substrate or both. Suramin does not inhibit pepsin at a pH of 1 to 2. In $1{\cdot}75 \times 10^{-4}$ M concentrations it is toxic to fumarase but not to urease at pH 7·0 (Quastel, 1931). Acceleration of post-mortem lactic acid production in muscle and liver has been reported (Fürth *et al.*, 1932). On the other hand, blood glycolysis may be retarded (Stuber and Lang, 1926), though Tcherniakofsky and Nattan-Larrier (1937) could not confirm this finding.

Hyaluronidase is extremely sensitive to the drug, 85 per cent. inhibition of the enzyme being brought about by 7×10^{-5} M and 15 per cent. by 7×10^{-7} M (Beiler and Martin, 1948). Town *et al.* (1949) find that although suramin has no inhibitory action on urease at pH 7·5, yet considerable inhibition occurs at pH 5·0: at this pH a final concentration of $3{\cdot}3 \times 10^{-4}$ M suramin gives 90 per cent. inhibition and 7×10^{-5} M suramin inhibits to the extent of 25 to 65 per cent., the percentage varying with the time of contact of drug and enzyme. It is probable that suramin acts by competitive inhibition, which may be related to the presence of a urea structure in suramin, although the action of the drug on urease and other enzymes, like the combination with serum and other proteins and the persistence of the drug in the animal body, may be largely determined by the sulphonic acid groups of suramin. Town *et al.* (1949) find that whereas at *p*H 5 urease is strongly inhibited by suramin, invertase is not.

Suramin acts also on carbohydrate-metabolising enzymes. Thus in concentrations greater than $2 \cdot 0 \times 10^{-5}$ M it completely inhibits the fermentation of glucose by yeast juice at *p*H 6·0 to 7·0 : this concentration is less than that in the plasma of rabbits after a dose of the same concentration. The autofermentation of glycogen by yeast juice is also inhibited, but not so strongly. Of the individual yeast enzymes, Wills and Wormall (1949) find that hexokinase at *p*H 6·0 to 7·0 is inhibited to the extent of 80 or 90 per cent. by suramin in a dilution of M/20,000: yeast decarboxylase, on the other hand, is sensitive under the same conditions only to M/6,000.

Reactions of suramin with other enzymes *in vitro* are as follows :

Enzyme.	*p*H.	Concentration of suramin.	Percentage inhibition.
Succinic dehydrogenase .	7·4	M/1,000	95
Choline dehydrogenase .	7·4	M/5,000	40
Catalase	6·5	M/3,000	0
,, . . .	6·0	,,	0
Cytochrome oxidase . .	7·4	,,	0
Cholinesterase . . .	7·5	,,	0
Tyrosinase . . .	6·0	,,	0
Arginase	8·9	,,	0
D-Amino-acid oxidase . .	7·4	,,	0

Enzyme inhibition by suramin is of two types : that occurring at *p*H 7·0 to 7·5 and that occurring only at more acid reactions. Since hexokinase and some other enzymes are inhibited by suramin in concentrations and under conditions which are similar to those found *in vivo* after therapeutic injections of suramin, it is possible that the trypanocidal action of suramin is due in whole or in part to disturbance in the carbohydrate metabolism of the trypanosomes.

It is of interest that the suramin analogues studied by Spinks (1948) which persist in the blood stream also inhibit urease. If the sulphonic acid groups in suramin and its analogues react with basic groups in the tissue proteins and enzymes then the spatial arrangement of the sulphonic acid groups in the suramin molecule must be of considerable importance since simple aromatic acids do not appreciably inhibit urease.

Antrycide in concentrations as high as M/500 has no inhibitory effect on either urease, succinic dehydrogenase or the system of yeast juice enzymes required for the fermentation of glucose. The hydrolysis of trypsin is not significantly inhibited by M/2400 antrycide. Higher concentrations precipitate the substrate (Town *et al.*, 1949). Some of these enzymes are even mildly stimulated by high concentrations of antrycide.

(2) The Rate of Absorption and Excretion of Trypanocidal Substances by the Tissues

The rate of conversion of arsenical compounds into trypanocidally active substances, the penetration of these substances into the tissues and their rate of excretion is obviously closely correlated with chemotherapeutic action. Thus, as Cohen, King and Strangeways (1932b) point out, no apparent relation between the toxicity of arsonic acids for mice and the velocities of oxidation of the corresponding arsenoxides can be noted, since it is masked by the rate of excretion (Voegtlin and Thompson, 1922). The excretion of quinquevalent arsenicals injected into the blood stream has been studied in considerable detail by a number of observers, including Stühmer (1924), Röthermundt and Richter (1935), Richter (1937), Launoy and Fleury (1937). The last observers found that when rabbits were injected with such a quantity of an 8 per cent. solution of tryparsamide that between 0·7 and 2·66 mgm. of arsenic is given per gm. of blood of the injected animal, doses well within the therapeutic range, from 88 to 95 per cent. of the arsenic injected had disappeared within an hour. Three to four hours after injection only about 1 per cent. of the injected arsenic could be discovered in the blood, and after six hours only 0·1 to 0·4 per cent. Imponderable traces, however, could still be found twenty-nine hours after injection, provided from 10 to 15 ml. of blood were examined. It seems probable that these persistent traces represent arsenic which has first been fixed by the tissues, converted into the trypanocidal tervalent form, and again slowly liberated by the tissues. This is the view put forward by Murgatroyd, Russell and Yorke (1934), who, instead of determining the blood content of various arsenicals in terms of arsenic, studied the trypanocidal titre of the serum of rabbits after the intravenous injection of neoarsphenamine, reduced tryparsamide thioglycollate, and tryparsamide, drugs which are examples of arsphenamines, arylthioarsinites and quinquevalent arsenicals.

It was found that injection of neoarsphenamine and reduced tryparsamide conferred immediately on the serum a very high trypanocidal titre, which is proportional to the dose of the drug injected. This titre at once falls, at first quickly, and later more slowly, until it reaches zero. The only difference in the two compounds is that the fall in titre in the case of the reduced tryparsamide is much more rapid than in the case of the arsphenamine compound. The immediate effect of injection of a quinquevalent compound is to confer only a slight trypanocidal titre on the serum. Instead of falling, however, as happens with the other two drugs, the titre steadily rises, and attains its maximum only six hours after the injection. The maximum titre reached is, however, in no way comparable to the enormous titres obtained with the arsphenamines and tervalent compounds. These compounds obviously circulate unchanged and are rapidly eliminated from the blood stream. Tryparsamide, on the other hand, must be reduced to the tervalent form before it becomes trypanocidal, and as soon as this happens it is rapidly eliminated from the blood stream. The trypanocidal activity and the arsenic content of the cerebrospinal fluid in man after the administration of arsenicals have been examined by Hawking, Hennelly and Quastel (1937). After the intravenous injection of 3 gm. of tryparsamide the trypanocidal activity of the cerebrospinal fluid in man, as tested by the action of the cerebrospinal fluid on trypanosomes *in vitro*, becomes apparent after fourteen hours, rises to a maximum after between thirty and forty hours and diminishes to such an extent as to be too small to measure after eighty hours. After injection of the same dose, the total arsenic content, measured chemically, is at a maximum after fourteen hours and falls off during the next forty hours, results in agreement with those of Sicé, Cousin and Dantec (1933) who, after the last dose of a course of tryparsamide, detected traces of arsenic for from six to twelve hours after injection but not at later periods. The trypanocidal activity of the cerebrospinal fluid thus bears no definite relationship to the total amount of arsenic present, indicating that the ratio of arsenic existing in the trypanocidal (tervalent) form to the total arsenic present varies from patient to patient. The average values for the percentage of total arsenic present in the trypanocidally active form in the cerebrospinal fluid after intravenous injection of 3 gm. of tryparsamide are :—

After fourteen hours . . .	3 per cent.
After forty hours	18 ,, ,,
After sixty hours	5 ,, ,,

The proportion of active arsenic in the cerebrospinal fluid thus depends on (i) the rate of penetration of the original compound into the cerebrospinal fluid, (ii) the rate of conversion of the quinquevalent into the tervalent form and the diffusion of the latter into the cerebrospinal fluid, and (iii) the rate of conversion of this active form into some inert substance by combination with tissue compounds. Orsanine is equal or superior to tryparsamide in producing trypanocidal activity.

While neocryl appears to be almost as active in the secondary stage of trypanosomiasis as tryparsamide, Hawking, Hennelly and Quastel (1937) were unable to determine the development of any trypanocidal activity in the cerebrospinal fluid, although neocryl undoubtedly penetrates into the central nervous system.

Intravenous injection of tervalent arsphenamines leads to the appearance of very low concentrations of arsenic in the cerebrospinal fluid, and the trypanocidal activity is slight or absent. This accords with the therapeutic inefficiency of those compounds in neurosyphilis and the secondary stage of sleeping sickness. These results are, however, in contradiction to those obtained by Cornwall, Bunker and Myers (1931), who found that arsenic penetrated into the cerebrospinal fluid in larger quantities after the intravenous injection of silver arsphenamine than after the injection of tryparsamide.

As with tryparsamide (Hawking, 1937), Wright and Peters

(1948), working with oxophenarsine hydrochloride, found little or no correlation between the arsenical level in the body fluids and the trypanocidal titre of these fluids. In the case of oxophenarsine the amino-phenol group is rapidly dissociated from the arsenic (Maren, 1949).

Twelve hours after injection the former had disappeared from the blood, but the arsenic level was at about 1 mgm. per ml. for at least forty-eight hours. A closely related phenylarsenoxide containing p-$CONH_2$ instead of p-OH has far greater stability and exists as such for twenty-four hours. In the case of oxophenarsine it seems probable that the resulting fractions are o-aminophenol and As_2O_3 or As_2O_5. The link between the aromatic ring and arsenic is essential for therapeutic utility in infectious diseases. However, Crawford and Levvy (1947) found no evidence that unsubstituted phenylarsenoxide is degraded to As_2O_3 or As_2O_5 in the rabbit.

It seems that m-amino-p-carbamylphenylarsenoxide is relatively stable and may circulate as such for twelve to twenty-four hours. It may thus be of use in trypanosomiasis. Side-chains also may play a part in stability *in vivo* as well as ensuring specificity in the treatment of experiments and human infections, the p-$(CH_2)_3COOH$ compound being active in trypanosomiasis and the p-$CONH_2$ compound in filariasis.

The persistence of suramin in the tissues is discussed on p. 405.

(3) The Action of Trypanocidal Drugs on Trypanosomes

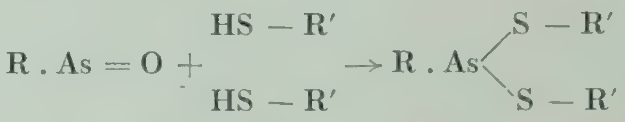
It is now realised that in drug-resistant trypanosomes the essential change is a decreased absorption of the drug by the parasite. Absorption of the drug by the trypanosome is thus seen to be the first essential for trypanocidal action. With certain trypanosomes the action of arsenicals can be explained by failure of absorption. Fischl and Singer (1935b), for instance, pointed out that *T. lewisi* absorbs arsenophenylglycine, which acts on the trypanosomes in considerable amount : the slightly active atoxyl is absorbed in much smaller amounts, and other aromatic arsenicals which do not kill *T. lewisi* are scarcely absorbed at all.

Although absorption of a certain quantity of the drug is necessary for curative effect, absorption is not synonymous with therapeutic action, for a number of substances, solganal and "sulfoharnstoff" (both gold-containing compounds), mepacrine, and rivanol, which are totally devoid of trypanocidal action, are as readily absorbed by trypanosomes as the trypanocidal dye trypaflavine (Singer and Fischl, 1935b).

The fact that mepacrine, although it has a photodynamic action on the trypanosomes, is without lethal action disposes of the view that photodynamic action is an essential part of trypanocidal activity (Fischl and Singer, 1935a; Singer and Fischl, 1935a), as does the fact that trypaflavine acts on trypanosomes in the dark.

So far as the quinquevalent arsenicals are concerned, there is some evidence to show that absorption is greater when reduction has taken place to the arsenoxide. Thus Fischl and Singer (1935a), for instance, found that when suspensions of *Spirochœta recurrentis*, *Proteus vulgaris*, erythrocytes, collodion, and animal charcoal are placed for one hour in a solution of 0·1 per cent. atoxyl they absorb much less arsenic than when the atoxyl has previously been digested with liver. Glutathione was found to increase the absorption of atoxyl by trypanosomes. The amount of arsenic actually fixed by trypanosomes has been calculated by Reiner, Leonard and Chao (1932), who estimated that if the volume of a trypanosome be taken as about 10^{-10} ml. and its surface area as 10^{-6} sq. cm., the average amount of arsenic bound would be 0·1 microequivalents for 10^{10} trypanosomes. If each molecule of the phenylarsenious oxide type occupies 300 sq. Å(3×10^{-4} sq. cm.) the surface of a single trypanosome should be occupied by 3×10^{7} molecules, arranged in a monomolecular layer. In fact, this is five to ten times the number of molecules actually fixed, and hence the amount of arsenic absorbed is sufficient to cover only about one-tenth of the surface of the trypanosome. If the trypanosomes are killed the amount of arsenic absorbed is of the order required to cover the surface of the trypanosome with a monomolecular layer.

When once absorption has occurred, and, in the case of a metallic compound, reduction or oxidation has produced the arsenoxide stage, a toxic action on certain substances in the body of the trypanosome follows. There is still, however, considerable uncertainty as to the mechanism by which the arsenic attacks trypanosomes. Ehrlich (1909 a, b) originally suggested that the toxic action of arsenicals might lie in their affinity for thiol groups. He was possibly influenced by an eighteenth-century controversy as to whether sulphur in the form of hepar sulphuris could be used as an antidote to arsenic (Majault, 1779). The suggestion was elaborated by Voegtlin, Dyer and Leonard (1923), who put forward the interesting hypothesis that, having arrived at the oxide stage, arsenic reacts with the reduced glutathione present in the tissues and the trypanosomes. In other words, the —SH group forms the chemo-receptor for arsenic.



$$\mathrm{R\,.\,As=O} + \begin{matrix}\mathrm{HS-R'}\\ \mathrm{HS-R'}\end{matrix} \rightarrow \mathrm{R\,.\,As}\Big\langle\begin{matrix}\mathrm{S-R'}\\ \mathrm{S-R'}\end{matrix}$$

In support of this view are the following facts :—

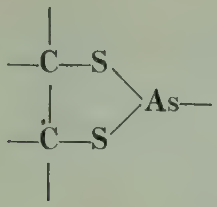
(1) By the nitroprusside reaction it is possible to show that trypanosomes contain an —SH group.

(2) If sodium thioglycollate is injected simultaneously with a minimum fatal dose of 3-amino-4-hydroxyphenylarsenoxide, a delay is noticed in the time of death of the animal.

(3) Feeding with glutamic acid and cysteine offers protection against a minimum fatal dose of 3-amino-4-hydroxyphenylarsenoxide administered three hours later.

(4) The injection of compounds containing an —SH group simultaneously with a trypanocidal drug slows the rate of disappearance of trypanosomes from an infected animal. Substances which act in this way include reduced glutathione, thioglycollic, thiolactic and thiosalicylic acids, and cysteine.

Whereas Voegtlin and his colleagues (1923) tentatively identified the —SH groups with glutathione, Rosenthal and Voegtlin (1930), and Schmitt and Skow (1935) later suggested that other —SH groups may be concerned, in particular, the "fixed" —SH groups of tissue proteins. Stocken and Thompson (1946) have shown that when arsenic reacts with keratin the ratio of combination with the sulphur of the protein is 1 As : 2 S, suggesting that a relatively stable ring of the type



is formed. It is therefore postulated that the sulphydryl-containing compound BAL detoxifies because of the removal of arsenic from the protein system and its incorporation *in vivo* into a more stable cyclic thioarsenite with BAL itself (Stocken and Thompson, 1946; Whittaker, 1947).

It hardly seems likely that —SH groups on the surface of the organisms are alone concerned in combining with arsenicals. Reiner, Leonard and Chao (1932) calculated that the amount of arsenic bound by trypanosomes in their experiments (0·1 microequivalent per 10^{10} organisms, or 6×10^{-6} molecules per trypanosome) is consistent with its combination with —SH groups on the surface of the organisms. Eagle (1945), however, using more active arsenicals in the shape of acid-substituted phenyl arsenoxides, found that twenty times as much arsenic had been bound (up to 30 μgm. by 2×10^9 organisms or $1{\cdot}2 \times 10^8$ molecules per trypanosome, with a surface area of approximately 10^{-6} sq. cm.). If all these —SH groups were on the surface of the organism, the mean distance between their points of attachment would be only 10^{-7} cm. This would imply that something of the order of every fifth atom on the surface of the trypanosome is an —SH group, or some other group equally reactive with arsenicals. Such a frequency far exceeds the analytical values for free —SH groups in proteins so far studied in this respect. An even greater disparity exists between the amount of arsenic bound and the known or probable density of —SH groups on the surface of the erythrocyte.

It is also a curious fact as pointed out by Eagle (1945) that even the least therapeutically active members of a series of ionised phenyl arsenoxides are nevertheless bound in some degree by trypanosomes since the trypanosomes contain a concentration of the drug several times in excess of that in the surrounding fluid although it exerts no deleterious effect on the trypanosomes.

If the degree of concentration of arsenic in the trypanosomes is due to the binding of the arsenoxides by —SH groups on the surface of the trypanosomes then these surface —SH groups cannot be very vital to the life of the trypanosome. This may be because they differ qualitatively from —SH groups in the interior of the cell or because they are too few when blocked to affect the vitality of the cell.

The analogy between chemotherapeutic interference and the action of the —SH group will be obvious.

Much criticism was at first directed against the view that the —SH group in trypanosomes represents the receptor for arsenic. Brown and Kolmer (1929), for instance, failed to establish any relation between the reduced glutiathione content of animal organs and the liability of various species to arsenic intoxication. Smythe and Reiner (1933) compared the action on glutathione of arsenicals and sodium mono-iodoacetate, a substance believed to inactivate glutathione. The results were difficult to interpret on the simple hypothesis of a direct inactivation of glutathione, since neither cysteine nor a thiosulphate solution inhibited the action of the iodoacetate either *in vivo* or *in vitro*. Cohen, King and Strangeways (1931a), however, as the result of the production of dithioarsinites by direct condensation of arylarsenious oxides with thiol compounds containing a carboxyl group, believed that there is full justification for the hypothesis that the lethal action of arsenic on living tissues is a chemical action and may well be an action on thiol groups and possibly on glutathione in particular.

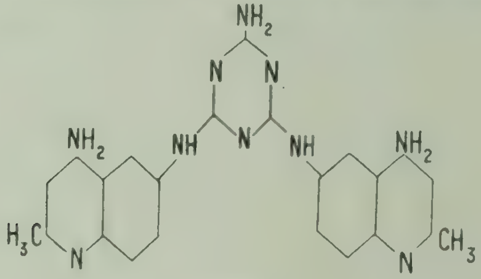
Chen *et al.* (1945) have emphasised the importance of cysteine, another —SH-containing compound, in relation to the chemotherapeutic action of tervalent antimonials. Cysteine also inactivates a number of antibiotics as well as quinones substituted in both the 2- and 3-positions (Page and Waller, 1946), and the antibacterial substances derived from the higher plants *Allium sativum*, *Arctium minus*, *Asarum canadense* (Cavallito and Bailey 1944 ; Cavallito *et al.*, 1946), and the radish (Ivánovics, 1948) The most active —SH compound in inactivating neoarsphenamine is, according to Simon (1948), L-cysteine hydrochloride, which is more effective than DL-methionine, sodium thioglycollate, and thiourea. Thus —SH-containing compounds are of importance in relation to the chemotherapeutic action of a number of substances having a considerable range of chemical constitution. Cavallito (1946), in discussing antibiotics, has noted that —SH groups may be inhibited in at least three ways: (*a*) adsorption by groups such as —NH_2 or —COOH in the vicinity of the —SH group so as to block the group physically; (*b*) adsorption in the vicinity of the —SH groups followed by a chemical reaction with the group; (*c*) no selective adsorption prior to rapid reaction. Klimek *et al.* (1948) show that antibacterial agents which are more selective as to the type of —SH group with which they react are more likely than non-selective agents to cause the development of resistant strains.

It has been suggested, however, by King and Strangeways (1942) that carboxyl-substituted arsenoxides differ from other arsenoxides in their mode of fixation on to, or of entry into, the trypanosome, although in both cases the ultimate lethal mechanism, presumably an inactivation of essential—SH groups, is the same for all arsenoxides.

Williamson and Lourie (1946) supported this view because of the finding that *p*-aminobenzoic acid (PABA) interferes with the trypanocidal action of butarsen whereas glutathione interferes with the action of both butarsen and mapharside. This same antagonistic action can be demonstrated *in vitro*, provided that the trypanosomes come into contact with PABA before they come into contact with butarsen. *In vivo* the antagonistic effect is greatly diminished if butarsen is injected first into mice, followed by PABA. Glutathione interferes with the trypanocidal action of butarsen *in vivo* as much as it does with that of oxophenarsine. PABA probably exerts an antagonistic effect by selectively limiting the admission of butarsen into or on to the trypanosomes: the underlying lethal mechanism for arsenical compounds, the interaction with sulphydryl groups, is not involved. Investigations by Williamson and Lourie (1947) show that arsenoxides of the type melarsen oxide differ from others in their mode of fixation on the trypanosome, but not in the essential mechanism of their lethal effect.

"Surfen C," bis(2-methyl-4-amino-6-quinolyl) melamine, contains the melamine nucleus and is active especially on *T.*

congolense and *T. brucei* (Iensch, 1937) but to some extent also on *T. rhodesiense.* Surfen C interferes with melarsen oxide just as *p*-aminobenzoic acid interferes selectively with butarsen. In addition *p*-aminobenzoic acid (PABA) interferes equally with the action of melarsen oxide and the other arsenoxides.



Surfen C

If it be agreed that interference with glutathione is evidence that the essential mechanism of the lethal action of arsenicals is by combination with —SH constituents of the parasite, it may be concluded that melarsen oxide and butarsen share this mode of action with other arsenoxides, but that they differ in their point of fixation on, or possibly point of entry into, the trypanosome, this point of fixation or entry being selectively blocked by Surfen C. It is reasonable to conclude that it is the melamine nucleus of Surfen C which selectively interferes with melarsen oxide since a " Surfen " derivative which differs from Surfen C in having a carbamide instead of a melamine linkage between the two quinoline systems (Iensch, 1937) gives no selective interference, although there is some degree of interference with oxophenarsine, butarsen and melarsen oxide. In addition, melamine itself interferes with the therapeutic action of melarsen oxide, but not with that of oxophenarsine or butarsen.

Thus the ultimate action of all the arsenoxides would appear to be essentially the same, namely, the inactivation of —SH groups, perhaps by the pyruvate oxidase system as suggested by Peters *et al.* (1945); melarsen oxide, however, has a different point of fixation or route of entry into the trypanosome. It is this difference which probably underlies the fact that melarsen oxide is active against trypanosomes resistant to the more usual type of arsenical.

Williamson and Lourie (1947) suggested that a similar explanation may hold good for the fact that the earlier pyrimidine prototypes of proguanil, such as 3349, are active against a proguanil-resistant strain of *Plasmodium gallinaceum.* The pyrimidine precursors of proguanil share with proguanil a common but unknown mode of lethal action on the malaria parasite, but differ in their mechanism of attachment to, or entry into, the plasmodial cell.

Further evidence of a possible *rôle* of PABA in the action of arsenicals was obtained by Schleyer and Schnitzer (1948), who showed that esters and amides of benzoic, *p*-hydroxybenzoic and *p*-aminobenzoic acids all antagonise the immobilisation of *T. equiperdum in vitro* by oxophenarsine hydrochloride. The action of another arsenoso compound, N-*p*-arsenosobenzyl-glycine amide hydrochloride, is similarly inhibited by the esters and amides of *p*-hydroxybenzoic acid, 2,5-dihydroxy benzoic acid, 2,4-dihydroxybenzoic acid, and nicotinic acid. The action of acriflavine on trypanosomes is likewise inhibited by the esters and amides of *p*-hydroxybenzoic and nicotinic acid. In all these instances the acids themselves are quite ineffective. The significance of these results is as yet uncertain : it is, however, known that arsenso compounds inhibit the oxidases of *Bacterium coli* and this inhibition is in turn abolished by methyl-*p*-hydroxybenzoate.

Whereas PABA interferes with the trypanocidal action of butarsen, it has no effect on the therapeutic activity of quinquevalent arsenicals, but it does seem to decrease the toxicity of such compounds. On the other hand it has but little action on the minimal lethal dose of tervalent arsenicals (Peters, 1943 ; Sandground and Hamilton, 1943a, b, c). Peters (1943) pointed out that whereas PABA does not interfere with the trypanocidal action of atoxyl on trypanosomes, it does inhibit the action of atoxyl on *Bacterium coli.* This suggests that the mode of action of atoxyl on bacteria and trypanosomes is different. Sulphanilamide and sulphamerazine, it may be noted, are without action on *T. equiperdum* in rats (Rosenthal and Bauer, 1941 ; Welch *et al.*, 1943).

Nevertheless, glutathione acts as a coenzyme to glyoxylase. This fact is of interest, since if arsenicals act by interfering with glutathione they must at the same time affect the carbohydrate metabolism of trypanosomes. This metabolism is very intense in the case of pathogenic trypanosomes, except *T. cruzi*, since in twenty-four hours a trypanosome consumes twice its own weight of sugar whereas the energy requirements of a 70-kgm. man for the same period are contained in about 500 gm. of sugar. It is of interest to note that *T. cruzi*, which does not possess this intense activity, is not acted on by arsenicals, and the non-pathogenic *T. lewisi*, which is also relatively unaffected by aromatic arsenicals, consumes only about a fifth of the sugar required by pathogenic, arsenic-susceptible trypanosomes. As Christophers and Fulton (1938a, b) have shown, the oxygen uptake of trypanosomes is dependent on a plentiful supply of glucose and, in addition, certain drugs inhibit oxygen consumption, the effect being in some degree parallel to therapeutic action. Other observers have suggested either that arsenic combines with the iron of the respiratory pigment or that some essential enzyme is attacked. Arsenic, in high dilution, for instance, exerts a toxic action on liver lipase and other enzymes (Clark, 1937). In this connection certain observations of Quastel (1931) are of interest. While the free naphthylamine disulphonic acids and their first *s*-carbamide derivatives are not trypanocidal and do not inactivate the enzyme fumarase, both actions begin at the second *s*-carbamide stage and are most marked at the third *s*-carbamide stage. A parallelism with the substantive properties of these derivatives to cotton fibres may be noted. Some structure must therefore apparently exist which is common to fumarase, trypanosomes, and cotton fibres, and this common factor must make for specific combination or absorption with the second and third *s*-carbamide derivatives of the naphthylamine-disulphonic acids. Glowazky (1937) also showed that when exposed to the action of *para*-aminophenylarsenoxide, trypaflavin- and " sulfoharnstoff "-resistant trypanosomes exhibit a quantitative change in the aerobic and a qualitative change in the anaerobic sugar metabolism after only thirty minutes : in the case of atoxyl these changes are noticeable only after six hours' exposure.

More precise information of the enzymes actually inhibited by arsenicals and the prevention of such inhibition has been furnished by the work of Barron and Singer (1943), and Gordon and Quastel (1947, 1948). It appears that —SH groups are present in a considerable number of enzymes.

Enzymes for Carbohydrate Metabolism. Organic arsenicals, as well as iodoacetamide and chloromercuribenzoic acids, interfere with pyruvate oxidation, as determined by the measurement of O_2 in liver slices; dismutation, as measured by CO_2 production in bicarbonate-Ringer and $N : CO_2$ as gas-phase; and condensation as shown by measurement of ketoglutarate, acetoacetate, acetyl methylcarbinol and carbohydrate synthesis. Glutathione reactivated all these reactions. In addition to pyruvate, the oxidation of malate and of ketoglutarate is inhibited by organic arsenicals and reactivated by glutathione (Barron and Singer, 1943). Gordon and Quastel (1947) also showed that the oxidation of pyruvate and possibly other ketonic acids is inhibited, the ketonic acids accumulating in considerable amounts. Chen (1948) likewise demonstrated that tryparsamide and oxophenarsine inhibit hexokinase, adenosine triphosphatase, and 3-phosphoglyceraldehyde dehydrogenase systems in lysed *T. equiperdum*. Similar results were obtained with the antimonial compound, stibamine.

Enzymes for Nitrogen Metabolism. Barron and Singer (1943) showed that D-amino acid oxidase, 2-glutamic acid oxidase, monoamine oxidase, and transaminase are all inhibited by tervalent arsenicals and reinactivated by glutathione. Diamine oxidase is not inhibited.

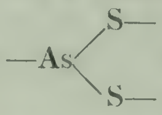
Urease is highly sensitive to tervalent arsenicals such as phenylarsenoxide, *m*-amino-*p*-hydroxyphenylarsenoxide, *p*-acetamidophenylarsenoxide and *m*-acetamido-*p*-carboxyphenylarsenoxide. Urease is also inhibited by the condensation products between phenylarsenoxide and thioglycollate and by reduced forms of atoxyl and tryparsamide: the two latter show little toxicity to urease until they are reduced. Urea does not protect urease against arsenicals.

Enzymes for Oxidation. Such reactions as the oxidation of choline by liver choline oxidase, as well as enzymes such as choline dehydrogenase, pyruvic acid oxidase, and lactate and glucose oxidases in brain tissue are inactivated by arsenicals. All these are probably thiol enzymes. Succinate, it may be noted, protects succinic dehydrogenase from the action of arsenoxides ; choline protects choline dehydrogenase to some extent.

Enzymes for Fat Metabolism. The presence of —SH groups is essential for the activity of the following enzymes concerned with fat metabolism : the oxidation of stearate by rat liver extract and the oxidation of β-hydroxybutyrate by animal tissues. Pancreatic lipase is partly inactivated by organic arsenicals.

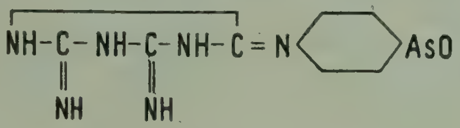
Esterases. Oxophenarsine, 3-amino-4-hydroxyphenyl arsine oxide ($6{\cdot}6 \times 10^{-5}$ M), causes a 57 per cent. inhibition of acetyl choline esterase : hog liver esterase is inactivated by 31 per cent. Enzymes containing no —SH groups essential for their activity are not inactivated by organic arsenicals : these enzymes include catalase, invertase, arginase, lactic oxidase, histaminase, carbonic anhydrase, acid phosphatase, pepsin, cytochrome oxidase and flavo-proteins.

Gordon and Quastel (1947) also confirm the fact that catalase, invertase, lactic dehydrogenase and cytochrome oxidase are not inhibited by tervalent arsenicals. Succinic dehydrogenase and choline esterase, on the other hand, are inhibited. Oxophenarsine hydrochloride has in fact been recommended as a test compound for the detection of thiol enzymes (Gordon and Quastel, 1948).

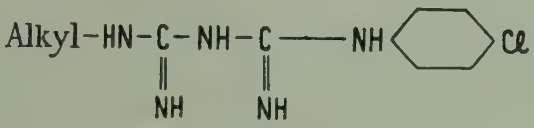
The toxicity of compounds of the type R . As . O to enzymes is neutralised by the addition of —SH compounds in excess : these —SH compounds condense with R . As . O . to form easily dissociated compounds and excess —SH is required to keep most of the —AsO in the form of the inert  $-\mathrm{As}\left\langle\begin{array}{l}\mathrm{S}-\\ \mathrm{S}-\end{array}\right.$ derivative : thus the reduction of quinquevalent arsenicals, such as tryparsamide, is probably accomplished in the body by means of —SH compounds such as glutathione. In addition to their effect on enzymes, compounds of the type R . As. O . inhibit vigorously the respiration of intact cells in the presence of glucose and sodium pyruvate.

Friedheim (1948) pointed out that in melarsen and melarsen oxide there exist similar structural carbon-nitrogen arrangements to those in pentamide and incidentally in proguanil. The significance of this arrangement for parasiticidal action on protozoa is unknown.

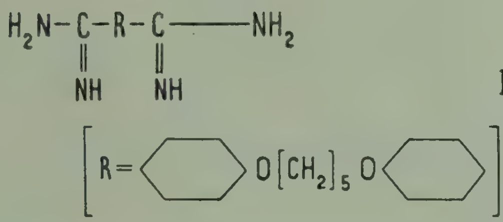
The mode of action of antimonials on trypanosomes has received relatively little study. It appears highly probable that quinquevalent antimonials exert their trypanocidal activity only after reduction to the tervalent form, but absolute proof is lacking.



Melarsen oxide



Proguanil



Pentamidine

Just as quinquevalent antimonials show a lag in therapeutic action when compared with tervalent compounds, so too compounds like stibamine show a lag in inhibiting the glucose metabolism of trypanosomes.

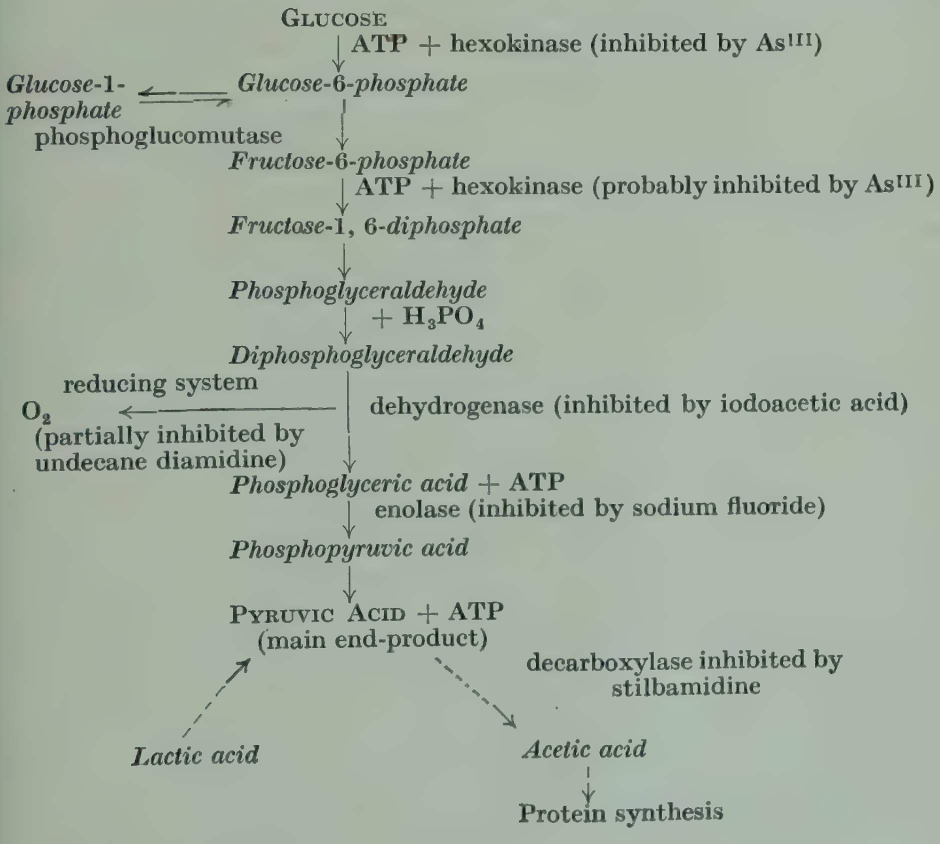
In vitro, the inhibitory effect of ter- and quinquevalent antimonials on the glucose metabolism of *T. equiperdum* is antagonised by cysteine hydrochloride at *p*H 7·5 (Chen and Geiling, 1948).

BAL and other —SH-containing compounds also inhibit the trypanocidal action of antimony compounds.

Even greater uncertainty still prevails in regard to the action of non-metallic compounds, more especially suramin. von Jancsó and von Jancsó (1935), however, have suggested that suramin produces a toxic inhibition of the sugar metabolism of the trypanosomes and that in this condition of " sugar-hunger " the trypanosomes are more readily phagocytosed by the reticulo-endothelial system. von Issekutz (1933) had previously shown that when infected animals are treated with suramin and their blood withdrawn a few hours later the contained trypanosomes exhibit diminished glycolysis *in vitro*. The effect of suramin on enzymes is discussed on p. 537.

Carbohydrate Metabolism and Trypanocidal Drugs. Although arsenical compounds appear to act by interfering with the sulphydryl group in trypanosomes, it has long been recognised that trypanosomes are dependent on an adequate supply of oxygen (Yorke, Adams and Murgatroyd, 1929 ; Geiger *et al.*, 1930 ; von Brand, 1933). *T. cruzi*, especially, has a high rate of utilisation of oxygen, 42·5 c.mm. of oxygen per 100 million organisms per hour (von Brand *et al.*, 1946 ; Chang, 1948). The end-products of carbohydrate metabolism have been variously described. Reiner and Smythe (1934) found the end point of the glucose metabolism of *T. equiperdum* to be pyruvic acid, Reiner *et al.* (1936) demonstrated that *T. lewisi* metabolised glucose more completely to yield formic, acetic, and succinic acids, ethyl alcohol, and carbon dioxide. Fulton and Stevens (1945) reported succinic, pyruvic, lactic, acetic and formic acids, glycerol, ethyl alcohol, and carbon dioxide. Marshall (1948) showed that *T. evansi* metabolises glucose mainly to pyruvic acid. The respiratory activity of *T. rhodesiense* was found by Christophers and Fulton (1938a) to depend on the presence of glucose : they also demonstrated the presence of dehydrogenase systems in trypanosome metabolism and their failure to undergo inhibition by cyanide. Reiner and Smythe (1934) reported very little carbon dioxide formation in the absence of bicarbonate, while Searle and Reiner (1940, 1941) pointed out the importance of carbon dioxide as an activator of anaerobic glycolysis in trypanosomes. Trypanosomes are able to induce glucose phosphorylation, since lysed trypanosomes can transform glucose to fructose-1, 6-diphosphate and triose phosphates, and oxidise phosphoglyceraldehyde to phosphoglyceric acid. Marshall (1948) showed that with *T. evansi* the phosphorylated intermediates present indicate that the intermediate metabolism of the parasites follows the typical Embden-Meyerhof-Parnas scheme characteristic of yeast and muscle metabolism. Marshall (1948) has therefore been able to construct a scheme showing the glucose metabolism of *T. evansi* and the points at which trypanocidal drugs may play a part in inhibiting this metabolism. In the presence of phenylarsine oxide very little glucose is utilised by

T. evansi and adenosine triphosphate, ATP, accumulates. The failure to convert glucose into glucose-6-phosphate is in agreement with the findings of Dixon and Needham (1946) on the action of arsenical vesicants on the glucose metabolism of skin. Quinquevalent arsenicals show no inhibition of trypanosome respiration : this is in agreement with the view that these compounds must first be reduced *in vivo* to the tervalent state before becoming active. There is less certainty as to the point of action of the diamidines. The straight-chain compounds such as undecane diamidine differ in action from the aromatic compounds such as stilbamidine. Undecane diamidine behaves like cyanide in decreasing the amount of pyruvate formed but stilbamidine has the reverse effect of increasing pyruvate accumulation. Though stilbamidine causes changes in the levels of phosphorylated intermediates, it does not interfere to any extent with the utilisation of glucose or oxygen. Its trypanocidal action may therefore


GLUCOSE
ATP + hexokinase (inhibited by As^{III})
Glucose-1-*phosphate*
Glucose-6-*phosphate*
phosphoglucomutase
Fructose-6-*phosphate*
ATP + hexokinase (probably inhibited by As^{III})
Fructose-1, 6-*diphosphate*
Phosphoglyceraldehyde
+ H_3PO_4
Diphosphoglyceraldehyde
reducing system
O_2
(partially inhibited by undecane diamidine)
dehydrogenase (inhibited by iodoacetic acid)
Phosphoglyceric acid + ATP
enolase (inhibited by sodium fluoride)
Phosphopyruvic acid
PYRUVIC ACID + ATP
(main end-product)
decarboxylase inhibited by stilbamidine
Lactic acid
Acetic acid
Protein synthesis

be due to the inhibition of pyruvate metabolism causing ultimate suppression of growth. This is in agreement with the finding by Lourie and Yorke (1937) that synthalin shows trypanocidal activity *in vitro* only after at least twenty-four hours' incubation. The point of action of undecane stilbamidine is not clear, since it does not greatly influence intermediate glucose metabolism. It does, however, cause a 50 per cent. reduction in oxygen uptake so that its point of action may lie somewhere in the dehydrogenase system.

The mode of action of the aromatic diamidines has so far received very little attention.

Marshall (1948) suggested that stilbamidine inhibits decarboxylase, thus interfering with the metabolism of pyruvic acid. Before such an inhibitory effect can occur, however, the stilbamidine must be absorbed by the trypanosomes. That such an absorption occurs has been shown by Hawking and Smiles (1942) and Hawking (1944), using both fluorescence microscopy and chemical tests. The absorption of stilbamidine is less marked than in the case of acriflavine and tervalent arsenicals, and whereas dead trypanosomes absorb these latter compounds they do not absorb stilbamidine. Absorption during its early stages is reversible. It would seem that the site of absorption of stilbamidine on the trypanosomes differs from that for acriflavine and tervalent arsenicals. Examination by fluorescence microscopy shows that stilbamidine is not evenly distributed over the surface of the trypanosome but is concentrated in the blepharoplast and certain other granules ; the rest of the cytoplasm and the nucleus take up little or no fluorescent material (Hawking and Smiles, 1942). On the other hand 4-carbamyl-4′-amidinostilbene which has no trypanocidal action on *T. rhodesiense* or *T. congolense* is found by fluorescence microscopy to be diffusely distributed throughout the trypanosomes (Fulton and Goodwin, 1949).

It has been suggested that the behaviour of stilbamidine and other similar compounds may be correlated with their electro-chemical properties. Stilbamidine, as the salt of a strong base, is highly ionised in dilute solution and its activity on trypanosomes might be attributed to its kationic nature. It has, however, also been thought that the trypanocidal activity of long-chain amidines, guanidines and *iso*-thioureas is a property of the free bases liberated by hydrolysis. Acriflavine is a salt of a strong quaternary base which cannot act in solution except as a strong kation. Reduced tryparsamide, which resembles these other compounds so closely in its behaviour toward trypanosomes, is the sodium salt of a weak acid and in solution would function as an anion. There is, however, evidence (Strangeways, 1937) that in solution hydrolysis occurs at the arsenic-sulphur link with the production from reduced tryparsamide ($NH_2 . CO . CH_2 . NH . C_6H_4 . As(S . CH_2 . COONa)_2$) of the arsenoxide $NH_2 . CO . CH_2 . NH . C_6H_4 . As = O$. Such a molecule is feebly amphoteric with a weakly negative group (—AsO) and a weakly positive group (—NH—). Hence the action of this particular group of compounds cannot easily be fitted into any scheme of anionic and kationic types of antiseptics such as has been put forward by Albert (1942). Whereas the behaviour of stilbamidine and of acriflavine to the host is, in view of the high toxicity and rapid disappearance from the blood stream, typical of electro-positive substances, reduced tryparsamide behaves similarly in respect of its weak electro-negative charge. Great significance cannot therefore be ascribed to this fact.

A curious and striking feature of many non-arsenical compounds with trypanocidal action is that they are symmetrical molecules with two identically constructed cyclic units as in the diamidines, trypan red, suramin, surfen C, Bayer 7602 Ac, and the cinnoline derivatives. What the significance of this structure is for trypanocidal action is at present unknown.

Although much further work requires to be done on the mode of action of trypanocidal drugs, it is obvious that they all show the three stages postulated by Clark (1933) for the action of a drug on a cell or parasite: (1) fixation of the drug by cells, (2) secondary chemical reactions between the drug and cell constituents, and (3) biological response to injury which results either in the death of the parasite or in its injury so that it is destroyed by phagocytic cells.

The non-identity of drug resistance to arsenicals and to diamidines indicates that there are different points of fixation on the trypanosome. In the case of antimonials and arsenicals this point of fixation may be an —SH-containing molecule or the secondary chemical reactions between the drug and parasite may involve —SH-containing enzymes.

The action of trypanocidal drugs is closely bound up with the manner in which trypanosomes cause the death of their vertebrate hosts. The mode of action of trypanocidal drugs will in fact only be fully explained when a true explanation has been found of the cause of death in trypanosomiasis. At present the ultimate cause of death in experimental trypanosomiasis and in the natural human infection is still a matter of controversy. The more important theories suggested are :—

Theory.	Authors.
Mechanical obstruction . .	Andrews *et al.* (1930).
Formation of trypanotoxin .	Schilling and Rondoni (1913) Kligler *et al.* (1929).
Acidosis	Nierenstein (1908). von Brand *et al.* (1932).
Hypoglycæmia	Dubois (1926). Hoppe & Chapman (1947).
Anoxia	Scheff (1928). Scheff and Rabati (1938).
Excess of potassium in the serum	Zwemer & Culbertson (1939).

Full references are given by Scheff and Thatcher (1949). The suggestion by Zwemer and Culbertson (1939) that death is due to an accumulation of potassium ions in the blood stream has been disproved by the work of Ikejiani (1946a, b) and Scheff and Thatcher (1949). At present there is little evidence that trypanocidal drugs owe their activity to changes in the tissue-metabolism of the vertebrate host.

(4) Specific Relationship between Arsenical Compounds and Certain Trypanosome Infections

It has been known for some years that all species of trypanosome do not react in the same way to the same aromatic arsenicals. *T. lewisi* of rats, for instance, is relatively insusceptible to those organic arsenicals which are more or less effective against *T. equiperdum*, *T. brucei* and other species of trypanosomes actively pathogenic to rats. These trypanosomes, on the other hand, are relatively unaffected by arsenophenylglycine, which is quite active against *T. lewisi*. A number of other compounds have been found by Kuhs and Tatum (1937) to be useless in infections due to *T. equiperdum* but active against *T. lewisi* infections in rats: these compounds are 4-carboxymethoxyphenylarsenoxide, 4-glycine-N-phenylarsonic acid, 5-amino-2-β-hydroxyethylaminophenylarsonic acid, 3-amino-4-*n*-butylphenylarsonic acid, 4-arsonophenoxyacetic acid, 5-amino-2-N-amylaminophenylarsonic acid, γ-4-arsonophenoxypropanol, 3-amino-4-*n*-butylaminophenylarsonic acid, 4-arsonophenoxyacetophenone, 4-β-hydroxy-*n*-propoxyphenyl-arsonic acid. On the other hand, a few drugs are equally active in infections due both to *T. lewisi* and *T. equiperdum*. The nature of the side-chains and the chemotherapeutic indices of these three groups of compounds in infections due to *T. equiperdum*, *T. rhodesiense* and *T. lewisi*, respectively, are shown in the table.

NATURE OF SIDE-CHAINS AND CHEMOTHERAPEUTIC INDICES OF AROMATIC ARSENICALS IN INFECTIONS DUE TO *T. equiperdum*, *T. rhodesiense* AND *T. lewisi*. (Kuhs and Tatum, 1937.)

Group.	Chemotherapeutic indices in infections due to:			Nature of side-chains.	Position.
	T. equiperdum.	*T. rhodesiense.*	*T. lewisi.*		
I	8·7	—	0	$AsO_3H_2 . OCH_2CH_2 . OH$	1, 4
	10	—	0	$AsO_3H_2 . OCH_2COCH_3$	1, 4
	12	6	0	$AsO_3H_2 . NHCH_2 . CONH_2$	1, 4
	18	—	0	$AsO_3H_2 . NH_2NHC_2H_4OH$	1, 3, 4
	20	2	0	$AsO . NH_2 . OH$	1, 3, 4
	25	4	0	$AsO_3H_2 . NHC_2H_4OH$	1, 4
	30	—	0	$AsO_3H_2 . NH_2 . OCH_2CHOHCH_3$	1, 3, 4
	50	23	0	$AsO_3H_2 . NH_2 . OCH_2CH_2OH$	1, 3, 4
II	1·5	0	7	$As = As . 2(NHCH_2COOH)$	1, 4, 4
	0	0	6	$AsO . OCH_2 . COOH$	1, 4
	0	0	2	$AsO_3H_2NHCH_2 . COOH$	1, 4
	0	0	1·8	$AsO_3H_2 . NH_2 . NHC_2H_4OH$	1, 2, 5
	0	0	1·5	$AsO_3H_2 . NH_2 . C_4H_9$	1, 3, 4
	0	0	1·4	$AsO_3H_2 . OCH_2COOH$	1, 4
	0	0	1·2	$AsO_3H_2 . NH_2 . NHC_5H_{11}$	1, 2, 5
III	1·1	0	2·5	$AsO_3H_2 . OCH_2 . CH_2CH_2OH$	1, 4
	1·0	0	1·1	$AsO_3H_2 . NH_2 . NHC_4H_9$	1, 3, 4
	1·6	0	2·2	$AsO_3H_2 . OCH_2COC_6H_5$	1, 4
	8·3	0	1·3	$AsO_3H_2 . OCH_2CHOHCH_3$	1, 4
IV	0	0	0	$AsO_3H_2 . NHCH_2 . COOH$	1, 2

It will be noted that, of the compounds effective on *T. lewisi*, the majority possess carboxyl groups whereas, in those effective on *T. equiperdum*, there are no carboxyl groups but, instead, side-chains of a basic nature. Replacement of the OH radical of a carboxyl group by a radical such as the NH_2 or CH_3 changes the compound from effectiveness on *T. lewisi* to effectiveness on *T. equiperdum*. The reason why certain compounds are effective in infections due both to *T. lewisi* and *T. equiperdum* is not apparent. Compounds effective in *T. lewisi* infections possess aliphatic side-chains which are acidic in nature, while those compounds curing *T. equiperdum* possess side-chains of a basic nature. A shift in the aliphatic side-chain from the ortho to the para position with respect to the arsenical radical produces a marked increase in therapeutic efficiency for *T. lewisi*.

(5) Synergistic Action

The possibility of synergistic action of two unrelated chemicals on pathogenic trypanosomes has been investigated both *in vitro* and *in vivo*. von Jancsó and von Jancsó (1935) believe that suramin and trypaflavine exert a synergistic action which they explain by the following experiments. Whereas a normal strain of *T. brucei* suspended in a solution of suramin for thirty minutes at 37° C. fails to absorb the drug, a strain of *T. brucei* systematically treated with trypaflavine gradually increases in permeability for suramin and when fully trypaflavine-resistant is rendered completely avirulent when exposed to suramin. Launoy (1935) found that orsanine in doses of 1 mgm. cured only about 23 per cent. of mice infected with nagana, while with a dose of 1·4 mgm. of lithium antimonylthiomalate only a very small percentage of mice were sterilised; when the two drugs were given together 70 per cent. of mice were cured. Similarly, Browning and Gulbransen (1935) showed that tryparsamide and styryl 245, in doses which separately are only slightly active, when combined cure a high percentage of mice infected with *T. brucei*. This synergism may possibly be correlated with the fact that while tryparsamide is quickly absorbed and excreted the styryl compound is only slowly absorbed and acts very gradually. The trypanosomes may, therefore, be first damaged by tryparsamide and then acted on for a considerable period by the styryl compound.

(6) Spontaneous Changes in the Reaction of Strains of Trypanosomes to Drugs

It has long been recognised that, in man, strains of *T. rhodesiense* are relatively resistant to the action of aromatic arsenicals, and recently isolated strains maintained in mice exhibit a similar resistance. On the other hand, after a number of passages in mice, *T. rhodesiense* becomes highly sensitive to arsenicals. Repeated passage in guinea-pigs, however, does not lead to so rapid an increase in arsenic sensitivity. Strains of *T. brucei* may also exhibit a similar change in arsenic sensitivity. As pointed out by Browning and Gulbransen (1935), when recently introduced into, and of low virulence for mice, *T. brucei* is relatively resistant to various trypanocidal drugs, but when completely adapted to mice and its pathogenicity increased to a maximum, infected mice are readily cured. Murgatroyd, Yorke and Corson (1937) have also found that changes occurred in the sensitivity to arsenicals of a strain of *T. brucei* as a result of five years' maintenance in the laboratory. In addition to increased sensitivity to arsenicals, mouse passage strains exhibited changes in morphology, increased pathogenicity for mice, decreased pathogenicity for guinea-pigs, and loss of transmissibility by *Glossina morsitans*. The guinea-pig passage strain exhibited no change of morphology, a gradual increase in pathogenicity for guinea-pigs and mice, and eventually a marked increase in sensitiveness to arsenicals, though this last change appeared more slowly than in the mouse-passage strains. The guinea-pig strains likewise finally lost their capacity of being transmitted by *Glossina*, but in one strain this character persisted for four years, and so long as it remained transmissible by *Glossina* its original characters appeared to be preserved unchanged.

In connection with changes in the reaction of trypanosomes to drugs, the observations of Broom, Brown and Hoare (1936) are of considerable interest. It was found that the electric charge of trypanosomes in laboratory animals is not constant but may be either positive or negative. These differences in the sign of the charge are apparently due to relapses having taken place in the previous history of the strain, since the charge of a relapse strain is the reverse of the parent strain. Since it was also found that positively charged trypanosomes are considerably more susceptible *in vivo* to tryparsamide than negatively charged variants of the same species, it follows that in estimating the chemotherapeutic index of drugs on trypanosomes the sign of the charge of the trypanosomes must be taken into account. The sign of the charge can be determined by means of the salt concentration test devised by Broom, Brown and Hoare (1936).

(7) Immunity and Chemotherapeutic Action

Closely related to drug resistance, and to the mode of action of trypanocidal drugs, is the question of persisting immunity. Ehrlich and Shiga (1904) first demonstrated that mice cured of trypanosome infections were immune to reinfection with the homologous strains. Lourie and O'Connor (1937) showed that, for *T. brucei* and *T. rhodesiense* infections, immunity persisted for eight months and six and a half months respectively. Browning and Calver (1943) noted immunity of thirteen months' duration for a strain of *T. congolense*. Fulton and Lourie (1946) similarly carried out immunisation studies with a normal strain of *T. rhodesiense*, an atoxyl-fast strain and a suramin-fast strain as well as with *T. congolense*. Complete failure to become reinfected twenty-six weeks after treatment of a *T. rhodesiense* infection and twenty weeks after a *T. congolense* infection was noted.

When immunity eventually breaks down, it may be due to a change in the antigenic character of the trypanosomes between the time of treatment and that of the immunity tests or, on the other hand, the trypanosomes inoculated at the test may possess the faculty of acquiring an anti-serum-fast character. In this conception of the immune state its persistence or absence is related to the stability of the antigenic constitution of the trypanosomes rather than to the mouse's power of developing and retaining immunity. In support of this view is the lytic power of sera and the fact, originally demonstrated by Franke (1905) and Ehrlich (1909a, b), that trypanosomes of a relapse may be immunologically different from those of the first infection. Fulton and Lourie (1946) found that *T. congolense* is more labile antigenically than

T. rhodesiense, a fact which would prevent the host's acquired immunity from completing the eradication of an infection after non-sterilising courses of treatment. Calver (1945) demonstrated that relapse strains of *T. congolense* are antigenically heterogeneous.

Fulton and Lourie (1946) reported that there is no cross-immunity between a parent strain of *T. rhodesiense* and a strain which has been made suramin-resistant, whereas there is complete immunity between a suramin-resistant and a atoxyl-resistant strain. This agrees with the conception that resistant strains are essentially relapse strains which are known to differ immunologically from the parent strain. Calver (1945) emphasised the antigenic lability of a mouse-passaged strain of *T. congolense* where the trypanosomes undergo immunological changes when an infection passes from the " fastigium," or acme, when trypanosomes are swarming in the blood, to the chronic stage of infection, when they are scanty in the blood stream. Browning and Calver (1943) and Browning *et al.* (1948) have noted that whereas with one strain animals were refractory for more than a year after cure, with another only half the animals resisted reinoculation after a recent cure. Animals cured of infection with a chronic variant of the Busimbi strain are not immune to the corresponding acme strain (Adamson, 1949). In the course of prolonged passages, however, the trypanosomes may revert immunologically to the condition of the original parent strain. A number of observers have observed this phenomenon (Mesnil and Brimont, 1909 ; Neumann, 1911 ; Braun and Teichmann, 1912 ; Rosenthal, 1913 ; Ritz, 1914 ; Lourie and O'Connor, 1937) ; it may occur in normal strains and possibly also in drug-fast strains, since Schilling (1929) showed that sometimes a drug-resistant strain is immunologically the same as the parent type. Schnitzer *et al.* (1946) brought forward evidence that the presence of antibody delays the appearance of a resistant strain of *T. equiperdum* against parafuchsin (pararosaniline hydrochloride). Passive immunisation with trypanocidal antiserum, no less than the active production of immune bodies, decreases the facility with which a resistant strain can be built up in mice by the splenectomy method. It is of some interest that a similar phenomenon is known to occur with pneumococci. A strain of pneumococcus can acquire resistance to sulphapyridine *in vivo* quite readily, but no such resistance develops if the compound is given in conjunction with sub-effective doses of type-specific antiserum.

The importance of the immune response on the part of the host in aiding and completing the chemotherapeutic action of drugs such as neoarsphenamine and antimonials such as melaminylphenylstibonic acid has been emphasised by Reiner and Leonard (1933) and Mayer and Brousseau (1946). Reiner and Chao (1933) showed that rats could be immunised against *T. equiperdum* by injections of trypanosomes killed by *p*-benzoquinone or neoarsphenamine. Strong agglutinins and lysins for the homologous strain could then be demonstrated *in vitro*. The immunity was strictly species specific. Whereas it was difficult to eradicate infection from rats by means of killed vaccines, nevertheless injections of vaccines together with very small subcurative doses of arsenicals did serve to eradicate *T. equiperdum* from rats. Similar results were obtained by Mayer and Brousseau with melaminylphenylstibonic acid in mice. The sera of such treated mice can be injected into normal mice and render them passively immune to injection with the homologous strain. Augustine (1943) believes that the main function of the immune body produced by a primary infection is to sensitise dividing trypanosomes, which are killed or immobilised and then phagocytosed by macrophages. Leonard (1946) would therefore claim that sublethal concentrations of a trypanocidal drug renders the trypanosomes more easily phagocytable, such phagocytosis being associated with an immunity response from the host.

Whereas agglutinins and trypanolysins have received most attention in the study of immune mechanisms, the work of Taliaferro (1941) has shown that the presence of trypanosomes in the blood stream stimulates the formation of another immune body, ablastin, the reproduction-inhibiting reaction product. Failure to develop the reproduction-inhibiting antibody, as shown by a prolongation of the reproductive phase, can be brought about in rats by a pantothenic acid deficiency in the host (Becker *et al.*, 1943, 1947) and by treating the host with sodium salicylate (Becker and Gallagher, 1947). Saul and Becker (1949) find that a daily dose of 45 mgm. of sodium salicylate per 100 gm. of body weight induces a prolonged reproduction period of *T. lewisi*. The prolongation of reproduction is due to interference either with the formation or action of ablastin. The effect of sodium salicylate on the chemotherapeutic action of trypanocidal drugs has not yet been investigated.

Some light on the mode of action of ablastin, which may be looked upon as an intrinsic chemotherapeutic agent, has been thrown by Moulder (1947), who finds that old, non-reproducing trypanosomes have a higher respiratory quotient and an increased sensitivity to malonate inhibition of oxygen uptake than young actively-dividing parasites. Ablastin thus appears to interfere with the respiratory mechanism.

When, however, trypanosomes such as *T. lewisi* are completely inhibited from reproducing by ablastin (Taliaferro, 1924, 1948), they are not removed from the peripheral blood stream by phagocytosis but remain in the blood of the rat for weeks or months. Eventual removal of the trypanosomes, though assumed by Regendanz and Kikuth (1927) and Regendanz (1932) to be due to non-specific phagocytosis, can be correlated with the appearance of specific trypanocidal antibodies (Coventry, 1930; Taliaferro, 1932, 1938).

(8) The *Rôle* of the Reticulo-endothelial System in the Chemotherapy of Trypanosomiasis

The *rôle* of the reticulo-endothelial system in the chemotherapeutic action of trypanocidal drugs still remains somewhat problematical. Kritschewski (1927a, b) claims that in mice splenectomy destroys the sterilising action of various trypanocidal drugs, but blockade of the reticulo-endothelial system does not. These results have been confirmed by Rubinstein (1928) in mice, and by Lisgunova (1928) in rats. Kritschewski (1928) has further shown that loss of the spleen is compensated for if, in the splenectomised animal, the drug is injected in combination with agar, so as to form a depot from which slow absorption may occur. Hasskó (1931) suggests that the decreased trypanocidal action of a number of chemotherapeutic agents in combined infections with trypanosomes and spirochætes is due to the fact that the latter damage the reticulo-endothelial system. Splenectomy in mice performed ten to twenty days before infection is known to reduce very considerably the resistance to *T. cruzi* (Wood *et al.*, 1948).

The chemotherapeutic action of suramin is also apparently closely bound up with an intact reticulo-endothelial system. von Jancsó and von Jancsó (1934 and 1935), whose results have been confirmed by Schlossberger and Grillo (1935), believe that suramin opsonises the trypanosomes by interfering with their carbohydrate metabolism. The slightly damaged trypanosomes are thus rendered more suitable for phagocytosis by the reticulo-endothelial cells of the liver, spleen, and bone marrow.

Although suramin can produce a cure in mice that have been splenectomised and have had their reticulo-endothelial cells blockaded by injections of a colloidal copper preparation, its chemotherapeutic index is then only 1 : 135 compared with 1 : 170 in the normal animal. In addition, in normal animals the time required for complete sterilisation of the blood is from fifteen to twenty-four hours, whereas in the animals with damaged reticulo-endothelial systems the time is from twenty-five to forty-four hours, the same period as is necessary to kill the trypanosomes *in vivo*. In the normal animal treated with suramin, abnormal parasites are rarely seen in the blood because, as soon as degeneration begins, the parasites are removed from the circulation by phagocytes : in the blockaded animal abnormal parasites are extremely common.

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The Effect of Drugs on Trypanosomes in Tsetse Flies

A point of some interest is the question whether trypanosomes present in tsetse flies are affected by trypanocidal drugs present in the blood which the flies have taken up from animals or man. Roubaud (1910) fed *Glossina* infected with *T. dimorphon* on guinea-pigs treated with atoxyl and found that the intestine, but not the proboscis, of the flies was sterilised. Similarly, Rodhain and his collaborators (1913) could not sterilise the probosces of tsetse flies by feeding them on blood rendered trypanocidal by tartar emetic. Kleine and Fischer (1922), using suramin, found that *Glossina* fed on animals treated with this drug were still infective. Duke (1927 and 1928) obtained similar results. Duke (1913) believed nevertheless that arsenophenylglycine would destroy *T. gambiense* in the intestinal tract of *G. palpalis*, though the trypanosomes already present in the salivary glands were unaffected.

The question has been studied by van Hoof, Henrard and Peel (1937). They found that a meal of blood containing tryparsamide or suramin does not destroy *T. gambiense* within the intestine, proventriculus and salivary glands of *Glossina*. Even the salivary gland trypanosomes found in the proboscis of infective *Glossina* do not appear to be affected. A limited experiment suggests that the same is true for neoarsphenamine and possibly for the antimonial Sdt. 411. Nevertheless, a considerable proportion of tsetse flies thus treated, although they harbour trypanosomes in the salivary glands and appear to be infective, are no longer able to transmit the infection to guinea-pigs.

The prolongation of the incubation period in guinea-pigs, bitten by tsetses treated in this manner, confirms the view that the drug modifies the virulence of the trypanosomes despite the absence of morphological changes. A preliminary meal on blood containing tryparsamide or suramin does not influence the evolution of *T. gambiense* in the tsetse, unless the drug is present in very large amounts. Such preliminary meals of drug-containing blood do not cause the trypanosomes to become drug-resistant. A preliminary feed on tryparsamide-containing blood has no inhibitory effect on the development of tryparsamide-resistant trypanosomes in the tsetse, even though the dose of the drug given to the guinea-pig is extremely large in relation to the degree of resistance of the trypanosome. Disinfecting meals given to tsetse flies during the course of the developmental cycle of the trypanosomes give results similar to those obtained with isolated infective flies. The frequency of infection is not lessened but transmission of the infection to guinea-pigs is delayed or inhibted. Analogous results were obtained with *T. brucei*, and *T. cazalboui* in tsetse flies given a suramin meal was notably less capable of causing infections. *T. congolense* appeared to be quite impervious to the effect of a drug meal.

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